

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
15 February 2001 (15.02.2001)

PCT

(10) International Publication Number
WO 01/011026 A1

(51) International Patent Classification⁷: C12N 9/02,
C12Q 1/26, C07K 16/00, C12N 5/10, C12Q 1/68, G01N
33/68, C12N 15/62, A61K 48/00, 38/43

(21) International Application Number: PCT/US00/19565

(22) International Filing Date: 4 August 2000 (04.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/147,601 6 August 1999 (06.08.1999) US

(71) Applicant (for all designated States except US): G.D.
SEARLE & CO. [US/US]; Corporate Patent Department,
P.O. Box 5110, Chicago, IL 60680-5110 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): GIERSE, James, K.
[US/US]; 141 Clara Avenue, St. Louis, MO 63119 (US).

(74) Agents: POLSTER, Rachel, A. et al.; G.D. Searle & Co.,
Corporate Patent Department, P.O. Box 5110, Chicago, IL
60680-5110 (US).

(81) Designated States (national): AE, AL, AM, AT, AU, AZ,
BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK,
DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL,
IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,
LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT,
RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,
UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

(48) Date of publication of this corrected version:
25 July 2002

(15) Information about Correction:
see PCT Gazette No. 30/2002 of 25 July 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CANINE CYCLOOXYGENASE-1 (COX-1) AND CYCLOOXYGENASE-2 (COX-2)

(57) Abstract: The present invention relates to genes that encode proteins of the canine cyclooxygenase-1 (COX-1) and the cyclooxygenase-2 (COX-2) enzymes, the canine COX-1 and canine COX-2 proteins, and assays for the evaluation of canine COX-1 and COX-2 activities. The invention also relates to nucleic acid molecules associated with these genes including their complements, homologues and fragments, and to methods of using these nucleic acid molecules, the enzymes and fragments of either.



CANINE CYCLOOXYGENASE -1 (COX-1) AND CYCLOOXYGENASE-2
(COX-2)

Field of the Invention

5 The present invention relates to genes that encode proteins of canine cyclooxygenase-1 (COX-1) and canine cyclooxygenase-2 (COX-2) enzymes, to canine COX-1 and canine COX-2 proteins, and to assays for the evaluation of canine COX-1 and COX-2 activities. The invention also relates to nucleic acid molecules associated with these genes and their complements, homologues and fragments, and to methods of using these nucleic acid molecules, the enzymes and fragments of either.

10

Background

Millions of dogs are kept either as pets or for utilitarian uses (e.g., seeing-eye dogs or guard dogs) (Olson and Moulton, *J Reprod Fertil Suppl* 47:433-8 (1993)). It is well known that chronic inflammatory processes such as degenerative joint disease
15 are common in canine species, ie. dogs, especially older dogs. There are, however, few drugs that can be successfully used in veterinary medicine for the treatment of inflammation (*Compendium of Veterinary Products*, Bennett 2d ed., 1993). Therefore, there is a large ongoing effort to find pharmaceutical agents which will impede or altogether stop the progress of such inflammatory disease processes in
20 dogs, or at least ameliorate the symptoms of the inflammation such as pain and edema.

Non-steroidal anti-inflammatory drugs, or NSAIDs, have been investigated extensively for anti-inflammatory activity in humans and in a more limited manner, in dogs. Only two NSAIDs have been approved for use in dogs by the Food and Drug
25 Administration, Committee on Veterinary Medicine (FDA/CVM), and this has resulted in substantial off-label use in dogs of NSAIDs approved for humans.

NSAID use in dogs has led to severe injury and even death due to gastrointestinal hemorrhage, perforation and peritonitis, and kidney and liver toxicity. Although NSAID toxicities are also seen in humans, they are more pronounced in dogs due to inappropriately high doses from a lack of dosing information, as well as the inherently
5 high degree of canine susceptibility to such adverse reactions. An alternative to NSAIDs, corticosteroids, has even more drastic side effects, especially when long term therapy is involved (MacDonald and Langston, Use of Corticosteroids and Non-steroidal Anti-inflammatory Agents, in: *Textbook of Veterinary Internal Medicine*, 284, Ettinger and Feldman, ed. (1995)).

10 The toxicity of NSAIDs is a result of their lack of specificity in prostaglandin inhibition. Prostaglandins, a group of compounds derived from arachidonic acid by the action of cyclooxygenase, are known to be involved in the inflammatory process. Some of these prostaglandins mediate inflammation; however, some of these prostaglandins also have a gastrointestinal protective effect. Previous NSAIDs were
15 found to prevent prostaglandin release by inhibiting the enzyme cyclooxygenase (COX) (Rubin and Papich, *Curr. Vet. Ther.* X:47-54 (1989)). The recent discovery of an inducible cyclooxygenase enzyme associated with the inflammation (cyclooxygenase II or COX-2), provides a viable target of inhibition which can effectively reduce inflammation and produce fewer and less drastic side effects than
20 occurs by targeting both COX-1 and COX-2 enzymes. It has been shown that the COX-2 isozyme is responsible for the production of prostaglandins exclusively or primarily at inflammatory sites. Drugs that selectively inhibit the human COX-2 isozyme relative to the human COX-1 isozyme can reduce inflammation without the side effects of gastrointestinal toxicity in humans.

25 For many years, the only known cyclooxygenase was the COX-1 isoform. Since its discovery, the inducible form (COX-2) has been cloned, sequenced, and characterized from chicken, sheep, murine, and human sources. These purified

enzymes have provided a means for assays and materials to identify and evaluate human pharmaceutical agents that selectively inhibit COX-2 relative to COX-1.

Summary of the Invention

A substantially-purified canine form of the COX-1 and COX-2 enzymes
5 would provide a means to carry out assays and materials valuable in identifying drugs that can reduce inflammation in dogs and which are without the toxic effects of current canine therapies. Thus, there is a substantial need in the art for the cloned, sequenced and characterized canine COX-1 and COX-2, and assays to screen and evaluate selective inhibitors of COX-2.

10 The present invention provides a substantially-purified nucleic acid molecule which specifically hybridizes to a nucleic acid molecule having a SEQ ID described herein or complement thereof, wherein the substantially-purified nucleic acid molecule encodes for a canine COX-2 protein or fragment thereof or complement of either. The present invention also provides a substantially-purified nucleic acid
15 molecule which specifically hybridizes to a SEQ ID described herein or complement thereof, wherein the substantially-purified nucleic acid molecule encodes for a canine COX-1 protein or fragment thereof or complement of either. Substantially-purified protein sequences of canine COX-1 and COX-2 are also disclosed. This invention also provides assays and materials to identify and evaluate veterinary agents that are
20 potent inhibitors of canine COX-2.

In one embodiment the present invention provides a substantially-purified nucleic acid molecule selected from the group of DNAs consisting of a nucleic acid molecule having SEQ ID: 1, the complement of the nucleic acid molecule having SEQ ID:1, an internal fragment of the nucleic acid molecule having SEQ ID: 1, and an
25 internal fragment of the complement of the nucleic acid molecule having SEQ ID: 1, wherein SEQ ID: 1 encodes a canine COX-1 protein or a fragment thereof.

In another embodiment the present invention provides a substantially-purified canine COX-1 nucleic acid molecule which comprises a nucleic acid sequence that is identical to at least 20 contiguous nucleotides of SEQ ID: 1 or a complement thereof.

In another embodiment the present invention provides a substantially-purified
5 canine COX-1 protein or a fragment thereof encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 1 or a complement thereof.

In another embodiment the present invention provides a substantially-purified canine COX-1 protein having SEQ ID: 2.

In another embodiment the present invention provides a substantially-purified
10 canine COX-1 protein or a fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 2.

In another embodiment the present invention provides an antibody capable of specifically binding to a substantially-purified canine COX-1 protein having SEQ ID: 2.

15 In another embodiment the present invention provides a substantially-purified nucleic acid molecule selected from the group of DNAs consisting of a nucleic acid molecule having SEQ ID: 3, a complement molecule of a nucleic acid molecule having SEQ ID: 3, an internal fragment a nucleic acid molecule having SEQ ID: 3, and an internal fragment of a complement molecule of a nucleic acid molecule having
20 SEQ ID: 3, wherein the SEQ ID: 3 encodes a canine COX-2 protein or a fragment thereof.

In another embodiment the present invention provides a substantially-purified canine COX-2 nucleic acid molecule which comprises a nucleic acid sequence that is identical to at least 20 contiguous nucleotides of SEQ ID: 3 or its complement.

25 In another embodiment the present invention provides a substantially-purified canine COX-2 protein or fragment thereof encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 4 or its complement.

In another embodiment the present invention provides a substantially-purified canine COX-2 protein having SEQ ID: 4.

In another embodiment the present invention provides a substantially-purified canine COX-2 protein or a fragment thereof wherein the protein or the fragment
5 thereof comprises at least 10 consecutive amino acids of SEQ ID: 4.

In another embodiment the present invention provides an antibody capable of specifically binding to the substantially-purified canine COX-2 protein having SEQ ID: 4.

In another embodiment the present invention provides a transformed cell
10 having a nucleic acid molecule which comprises an exogenous promoter region linked to a structural nucleic acid sequence, and a 3' non-translated sequence linked to the structural nucleic acid sequence, wherein the exogenous promoter region causes the production of a mRNA molecule; the structural nucleic acid sequence encodes a canine COX-1 gene or a fragment thereof; and the 3' non-translated sequence causes
15 termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

In another embodiment the present invention provides a transformed cell having a nucleic acid molecule which comprises an exogenous promoter region linked to a structural nucleic acid sequence, and a 3' non-translated sequence, wherein the
20 exogenous promoter region causes the production of a mRNA molecule; the structural nucleic acid molecule encodes a canine COX-2 gene or a fragment thereof; and the 3' non-translated sequence causes termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

In another embodiment the present invention provides a method for assaying a
25 canine COX-2 protein in a cell comprising (A) incubating a marker nucleic acid molecule with a complementary nucleic acid molecule obtained from the cell, wherein the incubation is performed under conditions permitting nucleic acid hybridization, and the marker nucleic acid molecule is capable of specifically hybridizing under high

stringency conditions to a canine COX-2-encoding nucleic acid molecule or a complement thereof; (B) hybridizing the marker nucleic acid molecule with the complementary nucleic acid molecule; and (C) assaying the complementary nucleic acid, wherein the assay is predictive of the level or pattern of the canine COX-2 protein.

In another embodiment the present invention provides a method for diagnosis or prognosis of a COX-2 related condition in a dog wherein the method comprises assaying the concentration of a molecule, the concentration being dependent upon the expression of a canine COX-2 gene, and the molecule being assayable in a biological sample from the dog.

In another embodiment the present invention provides a method for diagnosing a COX-2 related condition in a dog which comprises the steps of: (A) incubating under conditions permitting a nucleic acid hybridization a marker nucleic acid molecule with a complementary nucleic acid molecule obtained from a biological sample from the dog; (B) hybridizing the marker nucleic acid molecule with the complementary nucleic acid molecule; and (C) assaying the hybridized marker nucleic acid molecule and complementary nucleic acid molecule for a polymorphism; thereby diagnosing the COX-2 related condition in the dog wherein: the marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that specifically hybridizes to another polynucleotide that is linked to a canine COX-2 gene; and the polymorphism is predictive of a mutation affecting the canine COX-2 condition in the dog.

In another embodiment the present invention provides a method of determining an association between a polymorphism and a trait expressed by a dog wherein the method comprises: (A) hybridizing a nucleic acid molecule specific for the polymorphism to a gene in a cell, wherein the nucleic acid molecule has a nucleic acid sequence of SEQ ID: 3 or a complement thereof; and (B) determining the degree of association between the polymorphism and the trait.

In another embodiment the present invention provides a method of producing a cell containing an expressed canine COX-2 protein comprising: (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed

cell; thereby producing the cell containing the expressed canine COX-2 protein, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region, the structural region comprises a nucleic acid sequence of SEQ ID: 3, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and the functional nucleic acid molecule results in expression of the canine COX-2 protein.

In another embodiment the present invention provides a method of producing a cell exhibiting co-suppression of a canine COX-2 protein comprising: (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed cell; thereby producing the cell exhibiting co-suppression of the canine COX-2 protein; wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region, the structural region comprises a nucleic acid sequence of SEQ ID: 3, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in co-suppression of the canine COX-2 protein.

In another embodiment the present invention provides a method for causing co-suppression of a canine COX-2 protein in a transformed cell comprising: (A) transforming the cell with a nucleic acid molecule to produce a transformed cell; and (B) culturing the transformed cell; thereby causing co-suppression of the canine COX-2 protein in the transformed cell, wherein: the functional nucleic acid molecule comprises a promoter region; the promoter region is linked to a structural region, the structural region comprises a nucleic acid molecule having a nucleic acid sequence of SEQ ID: 3, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in co-suppression of the canine COX-2 protein.

In another embodiment the present invention provides a method of isolating a nucleic acid molecule that encodes a canine COX-2 protein or a fragment thereof

comprising (A) incubating under conditions permitting nucleic acid hybridization a first nucleic acid molecule comprising SEQ ID: 3 or the complement thereof with a complementary second nucleic acid molecule obtained from a cell; (B) hybridizing the first nucleic acid molecule with the second nucleic acid molecule; and (C)
5 isolating the second nucleic acid molecule.

In another embodiment the present invention provides a method of producing a cell containing an expressed canine COX-2 gene comprising (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed cell; thereby producing the cell containing the expressed canine COX-2 gene, wherein: the
10 functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region wherein the structural region comprises a nucleic acid molecule having a nucleic acid sequence consisting of SEQ ID:3 or complements thereof the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated
15 ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in overexpression of the canine COX-2 gene protein.

In another embodiment the present invention provides a method of producing a cell exhibiting co-suppression of a canine COX-2 gene comprising (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed
20 cell; thereby producing a cell co-suppression of a canine COX-2 gene, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region wherein the structural region comprises a nucleic acid sequence selected from the group consisting of SEQ ID: 3 or complements thereof, the structural region is linked to a 3' non-translated sequence that functions in the cell
25 to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in co-suppression of the canine COX-2 gene.

In another embodiment the present invention provides a method for causing co-suppression of a canine COX-2 gene in a cell comprising (A) transforming the cell
30 with a nucleic acid molecule; and (B) culturing the transformed cell; thereby reducing expression of a canine COX-2 gene in a cell, wherein the nucleic acid molecule has an

exogenous promoter region which causes the production of a mRNA molecule, the exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, the transcribed strand is complementary to a nucleic acid molecule having a nucleic acid selected from the group consisting of SEQ ID: 3 or complements thereof and the transcribed strand is complementary to an endogenous mRNA molecule, and the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule.

10 In another embodiment the present invention provides a substantially-purified nucleic acid molecule comprising a sequence which encodes a mammalian canine COX-2 protein.

In another embodiment the present invention provides a method of identifying a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises (A) contacting a test transformed cell with the test material, wherein the transformed cell expresses a gene encoding canine COX-2, and wherein the contacting is performed in the presence of test amount of prostaglandin precursor; (B) measuring the conversion of prostaglandin precursor to prostaglandin product by the test transformed cells; (C) contacting a control transformed cell with a control amount of prostaglandin precursor, wherein the control transformed cell expresses a gene encoding canine COX-2, and wherein the contacting is performed substantially in the absence of the test material; (D) measuring the conversion of prostaglandin precursor to prostaglandin product by the control transformed cells; and (E) comparing the amount of prostaglandin precursor converted to prostaglandin product by the test transformed cells to the amount of prostaglandin precursor converted to prostaglandin product by the control transformed cells. In a preferred embodiment, the prostaglandin precursor is arachadonic acid.

Brief Description of the Drawings

Fig. 1 diagrammatically sets forth a comparison between the amino acid
5 sequence which codes for canine COX-1 and the amino acid sequence which codes
for human COX-1.

Fig. 2 diagrammatically sets forth a comparison between the amino acid
sequences of canine COX-2 and human COX-2.

Fig. 3 diagrammatically sets forth a comparison between the amino acid
10 sequences of murine, rat, guineapig, rabbit, horse, canine, sheep, human and chicken
COX-2.

- SEQ NO: 1 – canine (*Canis familiaris*) COX-1 nucleic acid sequence
SEQ NO: 2 – canine (*Canis familiaris*) COX-1 amino acid sequence
15 SEQ NO: 3 – canine (*Canis familiaris*) COX-2 nucleic acid sequence
SEQ NO: 4 – canine (*Canis familiaris*) COX-2 amino acid sequence
SEQ NO: 5 – human (*Homo sapiens*) COX-1 nucleic acid sequence
SEQ NO: 6 – human (*Homo sapiens*) COX-1 amino acid sequence
SEQ NO: 7 – human (*Homo sapiens*) COX-2 nucleic acid sequence
20 SEQ NO: 8 - human (*Homo sapiens*) COX-2 amino acid sequence
SEQ NO: 9 - mouse (*Mus musculus*) COX-2 amino acid sequence
SEQ NO: 10 - rat (*Rattus norvegicus*) COX-2 amino acid sequence
SEQ NO: 11 - guinea pig (*Cavia porcellus*) COX-2 amino acid sequence
SEQ NO: 12 - rabbit (*Oryctolagus cuniculus*) COX-2 amino acid
25 sequence
SEQ NO: 13 - horse (*Equus caballus*) COX-2 amino acid sequence
SEQ NO: 14 - sheep (*Ovis aries*) COX-2 amino acid sequence

SEQ NO: 15 - chicken (*Gallus gallus*) COX-2 amino acid sequence

These detailed descriptions are presented for illustrative purposes only and are not intended as a restriction on the scope of the invention. Rather, they
5 are merely some of the embodiments that one skilled in the art would understand given the entire contents of this disclosure. All parts are by weight and temperatures are in Degrees centigrade unless otherwise indicated.

The following is a list of abbreviations and the corresponding meanings as
10 used interchangeably herein:

IMDM = Iscove's modified Dulbecco's media

mg = milligram

ml = milliliter

mL = milliliter

15 μ g = microgram

μ l = microliter

ODNs= oligonucleotides

PCR= polymerase chain reaction

RP-HPLC = reverse phase high performance liquid chromatography

20 ug = microgram

ul = microliter

The following is a list of definitions of various terms used herein:

The term "altered" means that expression differs from the expression response of cells
25 or tissues not exhibiting the phenotype.

The term "amino acid(s)" means all naturally occurring L-amino acids.

The term "chromosome walking" means a process of extending a genetic map by successive hybridization steps.

The term "complete complementarity" means that every nucleotide of one molecule is complementary to a nucleotide of another molecule.

The term "degenerate" means that two nucleic acid molecules encode for the same amino acid sequences but comprise different nucleotide sequences.

5 The term "exogenous genetic material" means any genetic material, whether naturally occurring or otherwise, from any source that is capable of being inserted into any organism..

10 The term "expression response" means the mutation affecting the level or pattern of the expression encoded in part or whole by one or more nucleic acid molecules.

The term "fragment" means a nucleic acid molecule whose sequence is shorter than the target or identified nucleic acid molecule and having the identical, the substantial complement, or the substantial homologue of at least 10 contiguous nucleotides of the target or identified nucleic acid molecule.

15 The term "fusion molecule" means a protein-encoding molecule or fragment that upon expression, produces a fusion protein.

The term "fusion protein" means a protein or fragment thereof that comprises one or more additional peptide regions not derived from that protein.

20 The term "marker nucleic acid" means a nucleic acid molecule that is utilized to determine an attribute or feature (*e.g.*, presence or absence, location, correlation, etc.) of a molecule, cell, or tissue.

The term "mimetic" refers to a compound having similar functional and/or structural properties to another known compound or a particular fragment of that known compound.

25 The term "phenotype" means any of one or more characteristics of an organism, tissue, or cell.

The term "probe" means an agent that is utilized to determine an attribute or feature (e.g. presence or absence, location, correlation, etc.) of a molecule, cell, tissue, or organism.

5 The term "promoter" is used in an expansive sense to refer to the regulatory sequence(s) that control mRNA production.

The term "protein fragment" means a peptide or polypeptide molecule whose amino acid sequence comprises a subset of the amino acid sequence of that protein.

The term "protein molecule" or "peptide molecule" means any molecule that comprises five or more amino acids.

10 The term "recombinant" means any agent (e.g., DNA, peptide, etc.), that is, or results from, however indirectly, human manipulation of a nucleic acid molecule.

The term "selectable or screenable marker genes" means genes whose expression can be detected by a probe as a means of identifying or selecting for transformed cells.

15 The term "specifically bind" means that the binding of an antibody or peptide is not competitively inhibited by the presence of non-related molecules.

The term "specifically hybridizing" means the quality of having a very high level of affinity for another material, for example, of one nucleic acid strand for a complementary strand having a high degree of homology.

20 The term "structural nucleic acid sequence" means a nucleic acid sequence that carries the coding for the amino acid sequence of a specific protein.

The term "substantial complement" means that a nucleic acid sequence shares at least 80% sequence identity with the complement.

25 The term "substantial fragment" means a fragment which comprises at least 100 nucleotides.

The term "substantial homologue" means that a nucleic acid molecule shares at least 80% sequence identity with another.

The term "substantially hybridizing" means that two nucleic acid molecules can form an anti-parallel, double-stranded nucleic acid structure under conditions (e.g. salt and temperature) that permit hybridization of sequences that exhibit 90% sequence identity or greater with each other and exhibit this identity for at least a
5 contiguous 50 nucleotides of the nucleic acid molecules.

The term "substantially purified" means that one or more molecules that are or may be present in a naturally occurring preparation containing the target molecule will have been removed or reduced in concentration.

The term "trait" means any genetically determined condition or state including
10 a healthy condition, a disease condition, or a passive condition.

Detailed Description of the Invention

I. Overview

The present invention stems in part from the isolation of cDNA molecules that
15 encode canine COX-1 and canine COX-2. SEQ ID: 1 sets forth the nucleic acid sequence of canine COX-1 and SEQ ID: 2 sets forth the amino acid sequence of canine COX-1. SEQ ID: 3 sets forth the nucleic acid sequence of canine COX-2 and SEQ ID: 4 sets forth the amino acid sequence of canine COX-2. Figure 1 diagrammatically sets forth a comparison between the amino acid sequences of human
20 COX-1 and canine COX-1. Figure 2 diagrammatically sets forth a comparison between the amino acid sequence which codes for human COX-2 and the amino acid sequence which codes for canine COX-2. Figure 3 diagrammatically sets forth a comparison between the nucleic acid sequences of human and canine COX-2, and their consensus sequence. Figure 4 sets forth a multiple sequence alignment of the cyclooxygenase-2
25 isozymes from mouse, rat, guinea pig, rabbit, horse, canine, sheep, human, and chicken.

II. Molecules of the Present Invention

The methods of the present invention are particularly useful in the monitoring of the expression of canine COX-2. The methods of the present invention are further useful in screening materials, compounds or compositions that selectively inhibit canine COX-2. The methods of the present invention are also relevant in the monitoring of the expression of canine COX-2 in canines with COX-2 related conditions, especially inflammatory joint disease and cancer. Molecules of the present invention are capable of being used to diagnose abnormal canine COX-2 expression. Molecules of the present invention can also be used as therapeutic agents.

- 10 The molecules of the present invention may be either naturally occurring or non-naturally occurring (i.e., synthetically prepared). As used herein, a naturally occurring molecule may be "substantially-purified," if desired, such that one or more impurities that are or may be present in a naturally-occurring preparation containing the molecule will have been removed or will be present at a lower concentration than that at which they would normally be found.

The molecules of the present invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of one nucleic acid molecule to hybridize to another nucleic acid molecule, or the ability of a protein molecule to be bound by an antibody molecule (or to compete with another molecule for such binding). Alternatively, such an attribute may be catalytic, and thus involve the capacity of the agent to mediate a chemical reaction or response.

The molecules of the present invention comprise nucleic acid molecules, proteins, and small organic compounds (i.e., having a molecular weight of less than about 2,000).

25 A. Nucleic Acid Molecules

A preferred class of agents of the present invention comprises canine COX-2 nucleic acid molecules. Such molecules may be either DNA or RNA.

In one embodiment, such nucleic acid molecules will encode all or a fragment of canine COX-2 protein, its "promoter," or its flanking gene sequences. The term "promoter" is used herein in an expansive sense to refer to the regulatory sequence(s) that control mRNA production. Such sequences include RNA polymerase binding sites, enhancers, etc. All such canine COX-2 molecules may be used in a diagnostic or therapeutic context.

Fragment canine COX-2 nucleic acid molecules may encode significant portion(s) of, or indeed most of, the canine COX-2 protein. Preferably, a fragment canine COX-2 nucleic acid molecule is identical or complementary to at least 20 contiguous nucleotides in SEQ ID: 3. More preferably it comprises at least 25 nucleotides in SEQ ID: 3. Even more preferably it comprises at least 50 nucleotides in SEQ ID: 3. More preferably still, it comprises at least 100 nucleotides in SEQ ID: 3. In a preferred embodiment, a fragment canine COX-2 nucleic acid molecule comprises at least one nucleotide that is not found in a corresponding position in canine COX-2.

Canine COX-2 nucleic acid molecules and fragment canine COX-2 nucleic acid molecules can specifically or selectively hybridize with other nucleic acid molecules. As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another when they have a very high level of affinity for one another, such as when one nucleic acid strand has a high degree of homology to a complementary strand. A nucleic acid molecule is the to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are the to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other.

Two molecules are the to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are the to be "complementary" if they can hybridize to one another with sufficient

stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook, *et al.*, *Molecular Cloning*, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, New York (1989). Conventional stringency

5 conditions are further described by Haymes, *et al.* *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985). Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure.

10 In another preferred embodiment, the fragment canine COX-2 molecule exhibits canine COX-2 enzymatic activity.

In one embodiment the present invention provides a substantially-purified nucleic acid molecule selected from the group of DNAs consisting of a nucleic acid molecule having SEQ ID: 1, the complement of the nucleic acid molecule having SEQ

15 ID:1, an internal fragment of the nucleic acid molecule having SEQ ID: 1, and an internal fragment of the complement of the nucleic acid molecule having SEQ ID: 1, wherein SEQ ID: 1 encodes a canine COX-1 protein or a fragment thereof.

Preferably, the the substantially-purified nucleic acid molecule encodes a canine COX-1 protein. Also preferably, the substantially-purified nucleic acid molecule has

20 SEQ ID: 1 or a complement thereof. In another embodiment of the present invention the substantially-purified nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-1 or complement thereof and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-1 or complement thereof. In yet another embodiment, the substantially-purified nucleic

25 acid molecule according to claim 4, wherein the nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-1 or complement thereof under high stringency conditions and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-1 or complement

thereof under high stringency conditions. In one preferred embodiment, the internal fragment of the nucleic acid molecule having SEQ ID: 1 contains at least 50 nucleic acid residues, more preferably at least 100 nucleic acid residues, more preferably still at least 200 nucleic acid residues, and still more preferably at least 500 nucleic acid residues.. In another preferred embodiment, the internal fragment of the complement of the nucleic acid molecule having SEQ ID: 1 contains at least 50 nucleic acid residues, more preferably at least 100 nucleic acid residues, more preferably still at least 200 nucleic acid residues, and still more preferably at least 500 nucleic acid residues.

10 In another embodiment, the present invention provides a substantially-purified canine COX-1 nucleic acid molecule which comprises a nucleic acid sequence that is identical to at least 20 contiguous nucleotides of SEQ ID: 1 or the complement of SEQ ID: 1. Preferably, the nucleic acid molecule comprises a nucleic acid sequence that is identical to at least 50 contiguous nucleotides of SEQ ID: 1 or a complement thereof. More preferably, the nucleic acid molecule comprises a nucleic acid sequence that is identical to at least 100 contiguous nucleotides of SEQ ID: 1 or a complement thereof.

The present invention also provides a substantially-purified nucleic acid molecule selected from the group of DNAs consisting of a nucleic acid molecule having SEQ ID: 3, a complement molecule of a nucleic acid molecule having SEQ ID: 3, an internal fragment a nucleic acid molecule having SEQ ID: 3, and an internal fragment of a complement molecule of a nucleic acid molecule having SEQ ID: 3, wherein the SEQ ID: 3 encodes a canine COX-2 protein or a fragment thereof. Preferably the substantially-purified nucleic acid molecule encodes the canine COX-2 protein. More preferably the substantially-purified nucleic acid molecule has the sequence described by SEQ ID: 3 or a complement thereof. In another embodiment the present invention provides a substantially-purified nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-2 or

complement thereof and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-2 or complement thereof. Preferably, the substantially-purified nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-2 or complement thereof under high stringency conditions and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-2 or complement thereof under high stringency conditions. In one preferred embodiment, the internal fragment of the nucleic acid molecule having SEQ ID: 3 contains at least 50 nucleic acid residues, more preferably at least 100 nucleic acid residues, more preferably still at least 200 nucleic acid residues, and still more preferably at least 500 nucleic acid residues. In another preferred embodiment, the internal fragment of the complement of the nucleic acid molecule having SEQ ID: 3 contains at least 50 nucleic acid residues, more preferably at least 100 nucleic acid residues, more preferably still at least 200 nucleic acid residues, and still more preferably at least 500 nucleic acid residues.

The present invention further provides a substantially-purified canine COX-2 nucleic acid molecule which comprises a nucleic acid sequence that is identical to at least 20 contiguous nucleotides of SEQ ID: 3 or a complement thereof. Preferably, the nucleic acid sequence that is identical to at least 50 contiguous nucleotides of SEQ ID: 3 or a complement thereof. More preferably the nucleic acid molecule comprises a nucleic acid sequence that is identical to at least 100 contiguous nucleotides of SEQ ID: 3 or its complement. In general terms, stringency conditions which promote DNA hybridization are, for example, 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50°C to a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high

stringency conditions at about 65°C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed.

In a preferred embodiment, a nucleic acid of the present invention will specifically hybridize to a nucleic acid molecule having SEQ ID: 3 or its complement under moderately stringent conditions, for example at about 2.0 x SSC and about 40°C.

In a particularly preferred embodiment, a nucleic acid of the present invention will specifically hybridize to SEQ ID: 3 or its complement under high stringency conditions. In one aspect of the present invention, a nucleic acid molecule of the present invention will comprise SEQ ID: 3 or its complement.

Fragment nucleic acid molecules can be determined and selected such that under specified conditions, such as high stringency, the fragment nucleic acid molecules can be used to specifically hybridize to canine COX-2 relative, for example, to canine COX-1.

In another aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 90% sequence identity with the nucleic acid sequence set forth in SEQ ID: 3 or its complement. In a preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 95% sequence identity with the nucleic acid sequence set forth in SEQ ID: 3 or its complement. In a more preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 98% sequence identity with SEQ ID: 3 or its complement. In the most preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 99% sequence identity with SEQ ID: 3 or its complement.

The degeneracy of the genetic code, which allows different nucleic acid sequences to code for the same protein or peptide, is known in the literature. (U.S. Patent No. 4,757,006). As used herein, a nucleic acid molecule is degenerate of

another nucleic acid molecule when the nucleic acid molecules encode for the same amino acid sequences, but comprise different nucleotide sequences. An aspect of the present invention is that the nucleic acid molecules of the present invention include nucleic acid molecules that are degenerate of SEQ ID: 3 and its complement.

5 Apart from their other uses such as those described below, the nucleic acid molecules of the present invention can be employed to obtain other canine COX-2 molecules. Nucleic acid molecules and fragments thereof of the present invention may be employed to obtain other nucleic acid molecules from dogs. Such nucleic acid molecules include the nucleic acid molecules that encode the complete coding
10 sequence of a protein and promoters and flanking sequences of such molecules. In addition, such nucleic acid molecules include nucleic acid molecules that encode for other isozymes or gene family members. Such molecules can be readily obtained by using the above-described nucleic acid molecules, or fragments thereof, to screen cDNA or genomic libraries obtained from species. Methods for forming such
15 libraries are well known in the art.

 Nucleic acid molecules and fragments thereof of the present invention may also be employed to obtain nucleic acid homologues. Such homologues include the nucleic acid molecule of other organisms (*e.g.*, monkey, cow, cat) including the nucleic acid molecules that encode, in whole or in part, protein homologues of plant
20 species or other organisms, sequences of genetic elements such as promoters and transcriptional regulatory elements. Methods for forming such libraries are well known in the art. Such homologue molecules may differ in their nucleotide sequences from those found SEQ ID: 3 or complement thereof. The nucleic acid molecules of the present invention therefore also include molecules that, although capable of
25 specifically hybridizing with the nucleic acid molecules may lack "complete complementarity."

In one embodiment, the nucleic acid molecules of the present invention include molecules that encode the canine COX-1 protein, its promoter, or flanking gene sequences. In a preferred embodiment, a canine nucleic acid of the present invention will specifically hybridize to a nucleic acid molecule having SEQ ID: 1 or its compliments. In one aspect of the present invention, a canine nucleic acid molecule of the present invention will comprise SEQ ID: 1 or its complement.

Fragment canine COX-1 nucleic acid molecules may encode significant portion(s) of, or indeed most of, the canine COX-1 protein. Preferably, a fragment canine COX-1 nucleic acid molecule is identical or complementary to at least 20 contiguous nucleotides in SEQ ID: 1. More preferably it comprises at least 25 nucleotides in SEQ ID: 1. Even more preferably it comprises at least 50 nucleotides in SEQ ID: 1. Most preferably, it comprises at least 100 nucleotides in SEQ ID: 1. In a preferred embodiment, a fragment canine COX-1 nucleic acid molecule comprises at least one nucleotide that is not found in a corresponding position in canine COX-1. In another preferred embodiment, the fragment canine COX-1 molecule exhibits canine COX-1 enzymatic activity.

In another embodiment of the present invention, a nucleic acid molecule of the present invention shares between 100% and 90% sequence identity with the canine COX-1 nucleic acid sequence set forth in SEQ ID: 1 or its complement. In a further aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 95% sequence identity with the canine nucleic acid sequence set forth in SEQ ID: 1 or its complement. In a more preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 98% sequence identity with SEQ ID: 1 or its complement. In a still more preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 99% sequence identity with SEQ ID: 1 or its complement.

The present invention also provides a substantially-purified canine COX-2 protein or fragment thereof encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 4 or its complement.

The present invention also provides a substantially-purified canine COX-1
5 protein having SEQ ID: 2.

The present invention also provides a substantially-purified canine COX-2 protein or fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 4.

The present invention also provides an antibody capable of specifically
10 binding to the substantially-purified canine COX-2 protein.

The present invention also provides a substantially-purified canine COX-2 protein or fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 4.

Nucleic acid molecules of the present invention may be used to find any
15 canine COX-2 nucleic acid homologue. Nucleic acid molecules of the present invention may also be used to obtain any mammalian COX-2 homologue.

Any of a variety of methods may be used to obtain one or more of the above-described nucleic acid molecules. The following individual references (each of which is individually herein incorporated by reference) each describes useful method of
20 obtaining nucleic acid molecules.

Zamechik et al., Proc. Natl. Acad. Sci. (U.S.A.) 83:4143-4146 (1986).

Goodchild et al., Proc. Natl. Acad. Sci. (U.S.A.) 85:5507-5511 (1988).

Wickstrom et al., Proc. Natl. Acad. Sci. (U.S.A.) 85:1028-1032 (1988).

25 Holt et al., Molec. Cell. Biol. 8:963-973 (1988).

Gerwitz et al., Science 242:1303-1306 (1988).

Anfossi et al., Proc. Natl. Acad. Sci. (U.S.A.) 86:3379-3383 (1989).

Becker et al., EMBO J. 8:3685-3691 (1989).

Automated nucleic acid synthesizers may be employed for this purpose. In lieu of such synthesis, the disclosed nucleic acid molecules may be used to define a pair of primers that can be used with the polymerase chain reaction to amplify and
5 obtain any desired nucleic acid molecule or fragment. Each of the following references (each of which is individually herein incorporated by reference) individually describe useful methods to define primers that can be used with the polymerase chain reaction.

- 10 Mullis et al., Cold Spring Harbor Symp. Quant. Biol. 51:263-273 (1986).
Erlich et al., European Patent 50,424.
European Patent 84,796.
European Patent 258,017.
European Patent 237,362.
- 15 Mullis, European Patent 201,184.
Mullis et al., U.S. Patent 4,683,202.
Erlich, U.S. Patent 4,582,788.
Saiki, R. et al., U.S. Patent 4,683,194.

The canine COX-2 promoter sequence(s) and canine COX-2 flanking
20 sequences can also be obtained using the SEQ ID: 3 sequence provided herein. In one embodiment, such sequences are obtained by incubating oligonucleotide probes of canine COX-2 oligonucleotides with members of genomic canine libraries and recovering clones that hybridize to the probes. In a second embodiment, methods of "chromosome walking," or 3' or 5' RACE may be used to obtain such sequences.

25 Each of the following individual references contains useful descriptions or "chromosome walking," or 3' or 5' RACE.

Frohman, M.A. et al., *Proc. Natl. Acad. Sci. (U.S.A.)* 85:8998-9002 (1988).

Ohara, O. et al., *Proc. Natl. Acad. Sci. (U.S.A.)* 86:5673-5677 (1989).

B. Proteins and Peptides

A second class of preferred agents comprises the canine COX-1 and COX-2 proteins, and their peptide fragments, fusion proteins, and analogs. Canine COX-1 and COX-2 proteins may be produced via chemical synthesis, or more preferably, by
5 expressing canine COX-1- or COX-2-encoding cDNA in a suitable bacterial or eukaryotic host. Most preferably, the subsequence of such cDNA that encodes canine COX-1 or COX-2 may be used for this purpose (SEQ ID: 1 or 3). Suitable methods for expression are described by Sambrook, J., *et al.*, (*Molecular Cloning, a Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor,*
10 New York (1989)), or similar texts.

A "canine COX-2 fragment" is a peptide or polypeptide the amino acid sequence of which comprises a subset of the amino acid sequence of canine COX-2 protein. Preferably a canine COX-2 fragment molecule is identical or complementary to at least one region which corresponds to a contiguous 10 amino acid sequence of
15 SEQ ID: 4. More preferably, the canine COX-2 fragment is identical or complementary to at least one region which corresponds to a contiguous 50 amino acids of SEQ ID: 4. Even more preferably, the canine COX-2 fragment is identical or complementary to at least one region which responds to a contiguous 100 amino acids of SEQ ID: 4. A canine COX-2 protein or fragment thereof that comprises one or
20 more additional non-canine COX-2 peptide regions is a "canine COX-2 fusion" protein. Such molecules may be derivatized to contain carbohydrate or other moieties (for example, keyhole limpet hemocyanin). As in the case of canine COX-2 protein, the fragments and fusions of the present invention are preferably produced via recombinant means.

25 The analogs of the canine COX-2 protein molecules comprise canine COX-2 proteins, fragments or fusions in which non-essential, nor relevant, amino acid residues have been added, replaced, or deleted. In other words, in analogs of canine COX-2 protein, no amino acids have been added, replaced or deleted which would

affect the activity of the analog protein relative to parent canine COX-2. An example of such an analog is the COX-2 protein of a non-canine species, such as a primate, a cow, a cat, and the like. Such analogs can readily be obtained by any of a variety of methods. Most preferably, as indicated above, the disclosed SEQ ID: 3 will be used
5 to define a pair of primers that may be used to isolate the canine COX-2-encoding nucleic acid molecules from any desired species. Such molecules can be expressed to yield canine COX-2 analogs by recombinant means.

Accordingly, in one embodiment the present invention provides a substantially-purified canine COX-1 protein or a fragment thereof wherein the canine
10 COX-1 protein or its fragment is encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 1 or a complement of SEQ ID: 1.

In another embodiment, the present invention provides a substantially-purified canine COX-1 protein having SEQ ID: 2.

In yet another embodiment, the present invention provides a substantially-purified
15 purified canine COX-1 protein or a fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 2. Preferably the substantially-purified canine COX-1 protein or a fragment thereof is a fusion protein.

The present invention further provides a substantially-purified canine COX-2 protein or a fragment thereof encoded by a nucleic acid sequence which specifically
20 hybridizes to SEQ ID: 4 or its complement. Preferably, the substantially-purified canine COX-2 protein or fragment specifically hybridizes to SEQ ID: 4.

Alternatively, the substantially-purified canine COX-2 protein or fragment thereof can specifically hybridize to the complement of SEQ ID: 4.

The present invention further provides a substantially-purified canine COX-2
25 protein having SEQ ID: 4. The present invention also provides a substantially-purified canine COX-2 protein or a fragment thereof wherein the protein or the fragment thereof comprises at least 10 consecutive amino acids of SEQ ID: 4. In one embodiment the canine COX-2 protein or fragment thereof is a fusion protein.

C. Antibodies Reactive Against canine COX-1 or canine COX-2

One aspect of the present invention concerns antibodies, single-chain antigen binding molecules, or other proteins that specifically bind to canine COX-2 protein and its analogs, fusions or fragments. Such antibodies are “anti-canine COX-2 antibodies,” and may be used, for example to measure canine COX-1 or canine COX-2 protein. As used herein, an antibody or peptide is the to “specifically bind” to canine COX-1 or canine COX-2 if such binding is not competitively inhibited by the presence of non-canine COX molecules.

10 Nucleic acid molecules that encode all or part of the canine COX-2 protein can be expressed, via recombinant means, to yield canine COX-2 protein or peptides that can in turn be used to elicit antibodies that are capable of binding canine COX-2. Such antibodies may be used in immunodiagnostic assays. Such canine COX-2-encoding molecules, or their fragments may be a “fusion” molecule (i.e. a part of a
15 larger nucleic acid molecule) such that, upon expression, a fusion protein is produced.

The antibodies that specifically bind canine COX-2 proteins and protein fragments may be polyclonal or monoclonal, and may comprise intact immunoglobulins, of antigen binding portions of immunoglobulins (such as (F(ab'), F(ab')₂) fragments, or single-chain immunoglobulins producable, for example, via
20 recombinant means. It is understood that practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of antibodies (see, for example, Harlow and Lane, *In Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1988)).

25 Murine monoclonal antibodies are particularly preferred. BALB/c mice are preferred for this purpose, however, equivalent strains may also be used. The animals are preferably immunized with approximately 25 µg of purified canine COX-2 protein

(r fragment thereof) that has been emulsified with a suitable adjuvant (such as TiterMax adjuvant (Vaxcel, Norcross, GA)). Immunization is preferably conducted at two intramuscular sites, one intraperitoneal site, and one subcutaneous site at the base of the tail. An additional i.v. injection of approximately 25 µg of antigen is preferably
5 given in normal saline three weeks later. After approximately 11 days following the second injection, the mice may be bled and the blood screened for the presence of anti-canine COX-2 antibodies. Preferably, a direct binding ELISA is employed for this purpose.

Most preferably, the mouse having the highest antibody titer is given a third
10 i.v. injection of approximately 25 µg of canine COX-2 protein, fragment, or fusion. The splenic leukocytes from this animal may be recovered 3 days later, and are then permitted to fuse, most preferably, using polyethylene glycol, with cells of a suitable myeloma cell line (such as, for example, the P3X63Ag8.653 myeloma cell line). Hybridoma cells are selected by culturing the cells under "HAT" (hypoxanthine-
15 aminopterin-thymine) selection for about one week. The resulting clones may then be screened for their capacity to produce monoclonal antibodies ("mAbs) to canine COX-2 protein, preferably by direct ELISA.

In one embodiment, anti-canine COX-2 monoclonal antibodies are isolated using canine COX-2 fusions, or conjugates, as immunogens. Thus, for example, a
20 group of mice can be immunized using a canine COX-2 fusion protein emulsified in Freund's complete adjuvant (approximately 50 µg of antigen per immunization). At three week intervals, an identical amount of antigen is emulsified in Freund's incomplete adjuvant and used to immunize the animals. Ten days following the third immunization, serum samples are taken and evaluated for the presence of antibody. If
25 antibody titers are too low, a fourth booster can be employed. Polysera capable of binding canine COX-2 at 1:5,000 dilution can also be obtained using this method.

In a preferred procedure for obtaining monoclonal antibodies, the spleens of the above-described immunized mice are removed, disrupted, and immune

splenocytes are isolated over a ficoll gradient. The isolated splenocytes are fused, using polyethylene glycol with BALB/c-derived HGPRT (hypoxanthine guanine phosphoribosyl transferase) deficient P3x63xAg8.653 plasmacytoma cells. The fused cells are plated into 96 well microtiter plates and screened for hybridoma fusion cells
5 by their capacity to grow in culture medium supplemented with hypoxanthine, aminopterin and thymidine for approximately 2-3 weeks. On average, out of every 10^6 spleen cells subjected to fusion yields a viable hybridoma. A typical spleen yields $5-10 \times 10^7$ spleen cells.

Hybridoma cells that arise from such incubation are preferably screened for
10 their capacity to produce an immunoglobulin that binds to canine COX-2 protein. An indirect ELISA may be used for this purpose. In brief, the supernatants of hybridomas are incubated in microtiter wells that contain immobilized canine COX-2 protein. After washing, the titer of bound immunoglobulin can be determined using, for example, a goat anti-mouse antibody conjugated to horseradish peroxidase. After
15 additional washing, the amount of immobilized enzyme is determined (for example, through the use of a chromogenic substrate). Such screening is performed as quickly as possible after the identification of the hybridoma in order to ensure that a desired clone is not overgrown by non-secreting neighbors. Preferably, the fusion plates are screened several times since the rates of hybridoma growth vary. In a preferred
20 embodiment, a different antigenic form of canine COX-2 may be used to screen the hybridoma. Thus, for example, the splenocytes may be immunized with one canine COX-2 immunogen, but the resulting hybridomas can be screened using a different canine COX-2 immunogen.

As discussed below, such antibody molecules or their fragments may be used
25 for diagnostic purposes. Where the antibodies are intended for diagnostic purposes, it may be desirable to derivatize them, for example with a ligand group (such as biotin) or a detectable marker group (such as fluorescent group, a radioisotope or an enzyme).

The ability to produce antibodies that bind canine COX-2 molecules permits the identification of mimetic compounds of canine COX-2. A "mimetic compound" of canine COX-2 is a compound that is not canine COX-2, or a fragment of canine COX-2, but which nonetheless exhibits an ability to specifically bind to anti-canine COX-2 antibodies. Such molecules can be used to elicit anti-canine COX-2 antibodies, and thus, may be used to assist diagnosis of canine COX-2 related disorders.

Accordingly, in one embodiment the present invention provides an antibody capable of specifically binding to a substantially-purified canine COX-1 protein having SEQ ID: 2; preferably the antibody is detectably labeled.

In another embodiment, the present invention provides an antibody capable of specifically binding to the substantially-purified canine COX-2 protein having SEQ ID: 4. Preferably the antibody is detectably labeled.

III. Uses of the Molecules of the Invention

In another embodiment the present invention provides plasmid DNA vectors for use in the expression of the canine COX-1 or COX-2 proteins. These vectors contain the DNA sequences described above which code for the polypeptides of the invention. Appropriate vectors which can transform eukaryotic cells, including mammalian cells and microorganisms capable of expressing the canine COX-1 or COX-2 proteins include expression vectors comprising nucleotide sequences coding for the canine COX-1 or COX-2 proteins joined to transcriptional and translational regulatory sequences which are selected according to the host cells used.

Vectors incorporating modified sequences as described above are included in the present invention and are useful in the production of the canine COX-1 or COX-2 polypeptides. The vector employed in the method also contains selected regulatory sequences in operative association with the DNA coding sequences of the invention

which are capable of directing the replication and expression thereof in selected host cells.

Transfer of a nucleic acid that encodes for a protein can result in expression or overexpression of that protein in a transformed cell. One or more of the proteins or fragments thereof encoded by nucleic acid molecules of the present invention may be expressed or overexpressed in a transformed cell. Particularly, any of the canine COX-1 or COX-2 proteins or fragments thereof may be expressed or overexpressed in a transformed cell. Such expression or overexpression may be the result of transient or stable transfer of the exogenous genetic material. "Exogenous genetic material" is any genetic material, whether naturally occurring or otherwise, from any source, which genetic material is capable of being inserted into an organism.

A construct or vector may include a promoter to express the protein or protein fragment of choice. Preferably, the promoter of the present invention is a COX-specific promoter. Promoters that can be used in the present invention include:

glucose-6 phosphatase promoter	Yoshiuchi <i>et al.</i> , <i>J. Clin. Endocrin. Metab.</i> 83:1016-1019 (1998)
interleukin-1 alpha promoter	Mori and Prager, <i>Leuk. Lymphoma</i> 26:421-433 (1997)
CMV promoter	Tong <i>et al.</i> , <i>Anticancer Res.</i> 18:719-725 (1998); Norman <i>et al.</i> , <i>Vaccine</i> 15:801-803 (1997)
RSV promoter	Elshami <i>et al.</i> , <i>Cancer Gene Ther.</i> 4:213-221 (1997); Baldwin <i>et al.</i> , <i>Gene Ther.</i> 4:1142-1149 (1997)
SV40 promoter	Harms and Splitter, <i>Hum. Gene Ther.</i> 6:1291-1297 (1995)

CD11c integrin gene promoter	Corbi and Lopez- Rodriguez, <i>Leuk. Lymphoma</i> 25:415-425 (1997)
GM-CSF promoter	Shannon <i>et al.</i> , <i>Crit. Rev. Immunol.</i> 17:301-323 (1997)
interleukin-5R alpha promoter	Sun <i>et al.</i> , <i>Curr. Top. Microbiol. Immunol</i> 211:173-187 (1996)
interleukin-2 promoter	Serfing <i>et al.</i> , <i>Biochim. Biophys. Acta</i> 1263:181-200 (1995) O'Neill <i>et al.</i> , <i>Transplant Proc.</i> 23:2862-2866 (1991)
c- fos promoter	Janknecht, <i>Immunobiology</i> 193:137-142 (1995) Janknecht <i>et al.</i> , <i>Carcinogenesis</i> 16:443-450 (1995) Takai <i>et al.</i> , <i>Princess Takamatsu Symp.</i> 22:197-204 (1991)
h-ras promoter	Rachal <i>et al.</i> , <i>EXS</i> 64:330-342 (1993)
DMD gene promoter	Ray <i>et al.</i> , <i>Adv. Exp. Med. Biol.</i> 280:107-111 (1990)

Promoters suitable for expression of the canine COX-1 or COX-2 proteins or fragments of either of the present invention in bacteria have been described by Hawley and McClure, *Nucleic Acids Res.* 11:2237-2255 (1983), Harley and Reynolds,
5 *Nucleic Acids Res.* 15:2343-2361 (1987) have also described promoters suitable for

expression of the canine COX-1 or COX-2 proteins or fragments of either of the present invention in bacteria. Such promoters include, for example:

the <i>recA</i> promoter-	Fernandez de Henestrosa <i>et al.</i> , <i>FEMS Microbiol. Lett.</i> 147:209-213 (1997) Nussbaumer <i>et al.</i> , <i>FEMS Microbiol. Lett.</i> 118:57-63 (1994) Weisemann <i>et al.</i> , <i>Biochimie</i> 73: 457-470 (1991)),
the Ptac promoter	Hasan <i>et al.</i> , <i>Gene</i> 56:141-151 (1987); Marsh, <i>Nucleic Acids Res.</i> 14:3603 (1986)
Ptac- <i>recA</i> hybrid promoter	

5 It is preferred that the particular promoter selected is capable of causing sufficient expression to result in the production of an effective amount of the canine COX-1 or COX-2 proteins or fragments of either to cause the desired phenotype.

Constructs or vectors may also include with the coding region of interest a nucleic acid sequence that acts, in whole or in part, to terminate transcription of that
10 region.

Translational enhancers may also be incorporated as part of the vector DNA. DNA constructs could contain one or more 5' non-translated leader sequences that may serve to enhance expression of the gene products from the resulting mRNA transcripts. Such sequences may be derived from the promoter selected to express the
15 gene or can be specifically modified to increase translation of the mRNA. Such regions may also be obtained from viral RNAs, from suitable eukaryotic genes, or from a synthetic gene sequence.

A vector or construct may also include a screenable marker. Screenable markers may be used to monitor expression. Exemplary screenable markers include:

β -glucuronidase encoded by the <i>uidA</i> gene (GUS)	Jefferson, <i>Plant Mol. Biol. Rep.</i> 5: 387-405 (1987) Jefferson <i>et al.</i> , <i>EMBO J.</i> 6: 3901-3907 (1987)
β -lactamase	Sutcliffe <i>et al.</i> , <i>Proc. Natl. Acad. Sci. (U.S.A.)</i> 75: 3737-3741 (1978)
luciferase	Clontech, Palo Alto, CA, USA Ow <i>et al.</i> , <i>Science</i> 234: 856-859 (1986)
β -galactosidase	Clontech, Palo Alto, CA, USA
GST	Stratagene)
Protein A	Calbiochem
blue fluorescent protein	Clontech, Palo Alto, CA, USA
fluorescent green protein	Clontech, Palo Alto, CA, USA

Included within the terms "selectable or screenable marker genes" are also genes which encode a secretable marker whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers which

5 encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes which can be detected by catalytic reactions. Secretable proteins fall into a number of classes, including small, diffusible proteins which are detectable, (e.g., by ELISA), small active enzymes which are detectable in extracellular solution (e.g., α -amylase, β -lactamase, phosphinothricin transferase), or proteins which are

10 inserted or trapped in the cell membrane (such as proteins which include a leader

sequence). Other possible selectable and/or screenable marker genes are apparent to those of skill in the art.

As another aspect of the present invention, there is provided a method for producing the canine COX-1 or COX-2 proteins. Suitable cells or cell lines may be mammalian, insect or yeast cells. For example, the canine COX-1 or COX-2 proteins or fragments of either of the present invention is expressed in a yeast cell, preferably *Saccharomyces cerevisiae*. The canine COX-1 or COX-2 proteins or fragments of either of the present invention can be expressed in *S. cerevisiae* by fusing it to the N-terminus of the URA3, CYC1 or ARG3 genes (Guarente and Ptashne, *Proc. Natl. Acad. Sci. (U.S.A.)* 78:2199-2203 (1981); Rose *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 78:2460-2464 (1981); and Crabeel *et al.*, *EMBO J.* 2:205-212 (1983)). Alternatively, the canine COX-1 or COX-2 proteins or fragments of either of the present invention can be fused to either the PGK or TRP1 genes (Tuite *et al.*, *EMBO J.* 1:603-608 (1982); and Dobson *et al.*, *Nucleic Acids. Res.* 11:2287-2302 (1983)). More preferably, the canine COX-1 or COX-2 proteins or fragments of either of the present invention are expressed as a mature protein (Hitzeman *et al.*, *Nature* 293:717-722 (1981); Valenzuela *et al.*, *Nature* 298:347-350 (1982); and Derynck *et al.*, *Nucleic Acids Res.* 11:1819-1837 (1983)).

Native and engineered yeast promoters suitable for use in the present invention have been reviewed by Romanos *et al.*, *Yeast* 8:423-488 (1992). Most preferably, the canine COX-1 or COX-2 proteins or fragments of either of the present invention is secreted by the yeast cell (Blobel and Dobberstein, *J. Cell Biol.* 67:835-851 (1975); Kurjan and Herskowitz, *Cell* 30:933-943 (1982); Bostian *et al.*, *Cell* 36:741-751 (1984); Rothman and Orci, *Nature* 355:409-415 (1992); Julius *et al.*, *Cell* 32:839-852 (1983); and Julius *et al.*, *Cell* 36:309-318 (1984)).

Where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g., V. A. Luckow, *Protein Eng.* J. L. Cleland., Wiley-Liss, New York, NY: 183-2180 (1996) and references cited therein. In addition, general

methods for expression of foreign genes in insect cells using baculovirus vectors are described in: O'Reilly *et al.*, *Baculovirus Expression Vectors: A Laboratory Manual*. New York, W.H. Freeman and Company (1992), and King and Possee, *The Baculovirus Expression System: A Laboratory Guide*, London, Chapman & Hall. An expression vector is constructed comprising a baculovirus transfer vector, in which a strong baculovirus promoter (such as the polyhedrin promoter) drives transcription of a eukaryotic secretion signal peptide coding region, which is translationally joined to the coding region for the multi-functional protein. For example, the plasmid pVL1393 (Invitrogen Corp., San Diego, California, U.S.A.) can be used. After construction of the vector carrying the gene encoding the multi-functional protein, two micrograms of this DNA is co-transfected with one microgram of baculovirus DNA into *Spodoptera frugiperda* insect cells, strain Sf9. Alternatively, recombinant baculoviruses can be created using a baculovirus shuttle vector system (Luckow *et al.*, *J. Virol.* 67: 4566-4579 (1993)), now marketed as the Bac-To-Bac™ Expression System (Life Technologies, Inc. Rockville, MD). Pure recombinant baculovirus carrying the multi-functional protein is used to infect cells cultured, for example, in Excell 401 serum-free medium (JRH Biosciences, Lenexa, Kansas) or Sf900-II (Life Technologies, Inc.). The multi-functional protein secreted into the medium can be recovered by standard biochemical approaches. Supernatants from mammalian or insect cells expressing the multi-functional proteins can be first concentrated using any of a number of commercial concentration units.

Most preferably, mammalian cells can be used to express the nucleic acid molecules of the present invention. Preferably, the nucleic acid molecules of the present invention are cloned into a suitable retroviral vector (*see, e.g.* Dunbar *et al.*, *Blood* 85:3048-3057 (1995); Baum *et al.*, *J. Hematother.* 5: 323-329 (1996); Bregni *et al.*, *Blood* 80:1418-1422 (1992); Boris-Lawrie and Temin, *Curr. Opin. Genet. Dev.* 3:102-109 (1993); Boris-Lawrie and Temin, *Annal. New York Acad. Sci.* 716:59-71 (1994); Miller, *Current Top. Microbiol. Immunol.* 158:1-24 (1992)), adenovirus

vector (Berkner, *BioTechniques* 6:616-629 (1988); Berkner, *Current Top. Microbiol. Immunol.* 158:39-66 (1992); Brody and Crystal, *Annal. New York Acad. Sci.* 716:90-103 (1994); Baldwin *et al.*, *Gene Ther.* 4:1142-1149 (1997)), RSV, MuSV, SSV, MuLV (Baum *et al.*, *J. Hematother.* 5: 323-329 (1996)), AAV (Chen *et al.*, *Gene Ther.* 5:50-58 (1998); Hallek *et al.*, *Cytokines Mol. Ther.* 2: 69-79 (1996)), AEV, AMV, or CMV (Griffiths *et al.*, *Biochem. J.* 241: 313-324 (1987)).

General methods and compositions for transforming a eukaryotic cell, bacteria and other microorganisms are known in the art (*see*, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1989)).

Technology for introduction of DNA into cells is well known to those of skill in the art. Four general methods for delivering a gene into cells have been described: (1) chemical methods (Graham and van der Eb, *Virology* 54:536-539 (1973)); (2) physical methods such as microinjection (Capecchi, *Cell* 22:479-488 (1980)), electroporation (Wong and Neumann, *Biochem. Biophys. Res. Commun.* 107:584-587 (1982); Fromm *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 82:5824-5828 (1985); U.S. Patent No. 5,384,253); and the gene gun (Johnston and Tang, *Methods Cell Biol.* 43:353-365 (1994)); (3) viral vectors (Clapp, *Clin. Perinatol.* 20:155-168 (1993); Lu *et al.*, *J. Exp. Med.* 178:2089-2096 (1993); Eglitis and Anderson, *Biotechniques*, 6:608-614 (1988)); and (4) receptor-mediated mechanisms (Curiel *et al.*, *Hum. Gen. Ther.* 3:147-154 (1992), Wagner *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 89:6099-6103 (1992)).

Transformation can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments (*see* for example Potrykus *et al.*, *Mol. Gen. Genet.* 205:193-200 (1986); Lorz *et al.*, *Mol. Gen. Genet.* 199:178 (1985); Fromm *et al.*, *Nature* 319:791 (1986); Uchimiya *et al.*, *Mol. Gen. Genet.* 204:204 (1986); Marcotte *et al.*, *Nature* 335:454-457 (1988)).

Assays for gene expression based on the transient expression of cloned nucleic acid constructs have been developed by introducing the nucleic acid molecules into cells by polyethylene glycol treatment, electroporation, or particle bombardment (Marcotte *et al.*, *Nature* 335: 454-457 (1988); McCarty *et al.*, *Cell* 66: 895-905 (1991); Hattori *et al.*, *Genes Dev.* 6: 609-618 (1992); Goff *et al.*, *EMBO J.* 9: 2517-2522 (1990)). Transient expression systems may be used to functionally dissect gene constructs.

In one embodiment, the canine COX-2 molecules of the present invention are used to determine whether an individual has a mutation affecting the level (i.e., the concentration of canine COX-2 mRNA or protein in a sample, etc.) or pattern (i.e., the kinetics of expression, rate of decomposition, stability profile, etc.) of the canine COX-2 expression (collectively, the "canine COX-2 Response" of a cell or bodily fluid) (for example, a mutation in the canine COX-2 gene, or in a regulatory region(s) or other gene(s) that control or affect the expression of canine COX-2), and being predictive of dogs who would be predisposed to, for example inflammatory joint disease, and other disorders. Such COX-2 related disorders include, but are not limited to, arthritis, rheumatoid arthritis, spondyloarthropathies, gouty arthritis, osteoarthritis, systemic lupus erythematosus and juvenile arthritis, auto-immune disease, allograft rejection, asthma, bronchitis, tendinitis, bursitis, and skin-related conditions such as psoriasis, eczema, burns, and dermatitis, post-operative inflammation including from ophthalmic surgery such as cataract surgery and refractive surgery, gastrointestinal conditions such as inflammatory bowel syndrome, and ulcerative colitis, neoplasia, such as colorectal cancer, and cancer of the breast, lung, prostate, bladder, cervix, and skin, vascular diseases, migraine headaches, periarteritis nodosa, thyroiditis, aplastic anemia, Hodgkin's disease, sclerodoma, rheumatic fever, type I diabetes, neuromuscular junction disease including multiple sclerosis, sarcoidosis, nephrotic syndrome, Behcet's syndrome, polymyositis, gingivitis, nephritis, hypersensitivity, conjunctivitis, swelling occurring after injury,

myocardial ischemia, myocardial infarction, ophthalmic diseases, such as retinitis, retinopathies, uveitis, ocular photophobia, and of acute injury to the eye tissue, allergic rhinitis, respiratory distress syndrome, endotoxin shock syndrome, atherosclerosis, pulmonary inflammation such as from viral and bacterial infections
5 and from cystic fibrosis, central nervous system disorders, such as cortical dementias including Alzheimer's disease, and central nervous system damage resulting from stroke, ischemia, and trauma.

As used herein, the canine COX-2 Response manifested by a cell or bodily fluid is the to be "altered" if it differs from the canine COX-2 Response of cells or of
10 bodily fluids of normal individuals. Such alteration may be manifested by either abnormally increased or abnormally diminished canine COX-2 Response. To determine whether a canine COX-2 Response is altered, the canine COX-2 Response manifested by the cell or bodily fluid of the canine is compared with that of a similar cell sample (or bodily fluid sample) of normal individuals. As will be appreciated, it
15 is not necessary to re-determine the canine COX-2 Response of the cell sample (or bodily fluid sample) of normal dogs each time such a comparison is made; rather, the canine COX-2 Response of a particular canines may be compared with previously obtained values of normal dogs.

In one sub-embodiment, such an analysis is conducted by determining the
20 presence and/or identity of polymorphism(s) in the canine COX-2 gene or its flanking regions which are associated with a disorder.

Any of a variety of molecules can be used to identify such polymorphism(s). In one embodiment, the canine COX-2 cDNA sequence (or a sub-sequence thereof) may be employed as a marker nucleic acid molecule to identify such
25 polymorphism(s). Alternatively, such polymorphisms can be detected through the use of a marker nucleic acid molecule or a marker protein that is genetically linked to (i.e., a polynucleotide that co-segregates with) such polymorphism(s). In a preferred aspect of this embodiment, such marker nucleic acid molecules will have the nucleotide

sequence of a polynucleotide that is closely genetically linked to such polymorphism(s). Polynucleotide markers that map to such locations are well known and can be employed to identify such polymorphism(s).

In an alternative embodiment, such polymorphisms can be detected through the use of a marker nucleic acid molecule that is physically linked to such polymorphism(s). For this purpose, marker nucleic acid molecules comprising a nucleotide sequence of a polynucleotide located within 1 mb of the polymorphism(s), and more preferably within 100 kb of the polymorphism(s), and most preferably within 10 kb of the polymorphism(s) can be employed.

The genomes of animals and plants naturally undergo spontaneous mutation in the course of their continuing evolution (Gusella, *Ann. Rev. Biochem.* 55:831-854 (1986)).

A "polymorphism" in the canine COX-2 gene or its flanking regions is a variation or difference in the sequence of the canine COX-2 gene or its flanking regions that arises in some of the members of a species. The variant sequence and the "original" sequence co-exist in the species' population. In some instances, such co-existence is in stable or quasi-stable equilibrium.

A polymorphism is thus the to be "allelic," in that, due to the existence of the polymorphism, some members of a species may have the original sequence (i.e. the original "allele") whereas other members may have the variant sequence (i.e. the variant "allele"). In the simplest case, only one variant sequence may exist, and the polymorphism is thus the to be di-allelic. In other cases, the species' population may contain multiple alleles, and the polymorphism is termed tri-allelic, etc. A single gene may have multiple different unrelated polymorphisms. For example, it may have a di-allelic polymorphism at one site, and a multi-allelic polymorphism at another site.

The variation that defines the polymorphism may range from a single nucleotide variation to the insertion or deletion of extended regions within a gene. In some cases, the DNA sequence variations are in regions of the genome that are

characterized by short tandem repeats (STRs) that include tandem di- or tri-nucleotide repeated motifs of nucleotides. Polymorphisms characterized by such tandem repeats are referred to as "variable number tandem repeat" ("VNTR") polymorphisms.

VNTRs have been used in identity and paternity analysis (Weber, U.S. Patent

- 5 5,075,217; Armour, *et al.*, *FEBS Lett.* 307:113-115 (1992); Jones, *et al.*, *Eur. J. Haematol.* 39:144-147 (1987); Horn, *et al.*, PCT Application WO91/14003; Jeffreys, European Patent Application 370,719; Jeffreys, U.S. Patent 5,175,082; Jeffreys, *et al.*, *Amer. J. Hum. Genet.* 39:11-24 (1986); Jeffreys, *et al.*, *Nature* 316:76-79 (1985); Gray, *et al.*, *Proc. R. Acad. Soc. Lond.* 243:241-253 (1991); Moore, *et al.*, *Genomics*
10 10:654-660 (1991); Jeffreys, *et al.*, *Anim. Genet.* 18:1-15 (1987); Hillel, *et al.*, *Anim. Genet.* 20:145-155 (1989); Hillel, *et al.*, *Genet.* 124:783-789 (1990)).

- The detection of polymorphic sites in a sample of DNA may be facilitated through the use of nucleic acid amplification methods. Such methods specifically increase the concentration of polynucleotides that span the polymorphic site, or
15 include that site and sequences located either distally or proximally to it. Such amplified molecules can be readily detected by gel electrophoresis or other means.

- The most preferred method of achieving such amplification employs the polymerase chain reaction ("PCR") (Mullis, *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* 51:263-273 (1986); Erlich *et al.*, European Patent Appln. 50,424; European
20 Patent Appln. 84,796, European Patent Application 258,017, European Patent Appln. 237,362; Mullis, European Patent Appln. 201,184; Mullis *et al.*, U.S. Patent No. 4,683,202; Erlich, U.S. Patent No. 4,582,788; and Saiki, *et al.*, U.S. Patent No. 4,683,194), using primer pairs that are capable of hybridizing to the proximal sequences that define a polymorphism in its double-stranded form.

- 25 In lieu of PCR, alternative methods, such as the "Ligase Chain Reaction" ("LCR") may be used (Barany, *Proc. Natl. Acad. Sci. (U.S.A.)* 88:189-193 (1991)). LCR uses two pairs of oligonucleotide probes to exponentially amplify a specific target. The sequences of each pair of oligonucleotides is selected to permit the pair to

hybridize to abutting sequences of the same strand of the target. Such hybridization forms a substrate for a template-dependent ligase. As with PCR, the resulting products thus serve as a template in subsequent cycles and an exponential amplification of the desired sequence is obtained.

5 LCR can be performed with oligonucleotides having the proximal and distal sequences of the same strand of a polymorphic site. In one embodiment, either oligonucleotide will be designed to include the actual polymorphic site of the polymorphism. In such an embodiment, the reaction conditions are selected such that the oligonucleotides can be ligated together only if the target molecule either contains
10 or lacks the specific nucleotide that is complementary to the polymorphic site present on the oligonucleotide. Alternatively, the oligonucleotides may be selected such that they do not include the polymorphic site (see, Segev, PCT Application WO 90/01069).

 The "Oligonucleotide Ligation Assay" ("OLA") may alternatively be
15 employed (Landegren, *et al.*, *Science* 241:1077-1080 (1988)). The OLA protocol uses two oligonucleotides which are designed to be capable of hybridizing to abutting sequences of a single strand of a target. OLA, like LCR, is particularly suited for the detection of point mutations. Unlike LCR, however, OLA results in "linear" rather than exponential amplification of the target sequence.

20 Nickerson, *et al.* have described a nucleic acid detection assay that combines attributes of PCR and OLA (Nickerson, *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:8923-8927 (1990)). In this method, PCR is used to achieve the exponential amplification of target DNA, which is then detected using OLA. In addition to requiring multiple, and separate, processing steps, one problem associated with such
25 combinations is that they inherit all of the problems associated with PCR and OLA.

Schemes based on ligation of two (or more) oligonucleotides in the presence of nucleic acid having the sequence of the resulting "di-oligonucleotide", thereby

amplifying the di-oligonucleotide, are also known (Wu, *et al.*, *Genomics* 4:560 (1989)), and may be readily adapted to the purposes of the present invention.

Other known nucleic acid amplification procedures, such as allele-specific oligomers, branched DNA technology, transcription-based amplification systems, or isothermal amplification methods may also be used to amplify and analyze such polymorphisms (Malek, *et al.*, U.S. Patent 5,130,238; Davey, *et al.*, European Patent Application 329,822; Schuster *et al.*, U.S. Patent 5,169,766; Miller, *et al.*, PCT appln. WO 89/06700; Kwoh, *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:1173 (1989); Gingeras, *et al.*, PCT application WO 88/10315; Walker, *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 89:392-396 (1992)). All the foregoing nucleic acid amplification methods could be used.

The identification of a polymorphism in the canine COX-2 gene can be determined in a variety of ways. By correlating the presence or absence of inflammatory joint disease in an dog with the presence or absence of a polymorphism in the canine COX-2 gene or its flanking regions, it is possible to diagnose the predisposition of an asymptomatic dog to inflammatory joint disease or other diseases. If a polymorphism creates or destroys a restriction endonuclease cleavage site, or if it results in the loss or insertion of DNA (e.g., a VNTR polymorphism), it will alter the size or profile of the DNA fragments that are generated by digestion with that restriction endonuclease. As such, dogs that possess a variant sequence can be distinguished from those having the original sequence by restriction fragment analysis. Polymorphisms that can be identified in this manner are termed "restriction fragment length polymorphisms" ("RFLPs"). RFLPs have been widely used in human and animal genetic analyses (Glassberg, UK Patent Application 2135774; Skolnick, M.H. *et al.*, *Cytogen. Cell Genet.* 32:58-67 (1982); Botstein, *et al.*, *Ann. J. Hum. Genet.* 32:314-331 (1980); Fischer, *et al.* (PCT Application WO90/13668); Uhlen, PCT Application WO90/11369).

In accordance with this embodiment of the invention, a sample DNA is obtained from a dog's cells. In a preferred embodiment, the DNA sample is obtained from the dog's blood. However, any source of DNA may be used. The DNA is subjected to restriction endonuclease digestion. Canine COX-2 is used as a probe in accordance with the above-described RFLP methods.

The polymorphism obtained in this approach can then be cloned to identify the mutation at the coding region which alters the protein's structure, or at the regulatory region of the gene which affects its expression level. Changes involving promoter interactions with other regulatory proteins can be identified by, for example, gel shift.

Several different classes of polymorphisms may be identified through such methods. Examples of such classes include: (1) polymorphisms present in the canine COX-2 cDNA of different dogs; (2) polymorphisms in non-translated canine COX-2 gene sequences, including the promoter or other regulatory regions of the canine COX-2 gene; (3) polymorphisms in genes whose products interact with canine COX-2 regulatory sequences; (4) polymorphisms in gene sequences whose products interact with the canine COX-2 protein, or to which the canine COX-2 protein binds.

In an alternate sub-embodiment, the evaluation is conducted using oligonucleotide "probes" whose sequence is complementary to that of a portion of canine COX-2 mRNA. Such molecules are then incubated with cell extracts of a patient under conditions sufficient to permit nucleic acid hybridization. For this sub-embodiment, cells of the trabecular meshworks are preferred. The detection of double-stranded probe-mRNA hybrid molecules is indicative of the presence of canine COX-2 mRNA; the amount of such hybrid formed is proportional to the amount of canine COX-2 mRNA. Thus, such probes may be used to ascertain the level and extent of canine COX-2 mRNA production in a patient's cells. Such nucleic acid hybridization may be conducted under quantitative conditions (thereby providing a numerical value of the amount of canine COX-2 mRNA present). Alternatively, the

assay may be conducted as a qualitative assay that indicates either that canine COX-2 mRNA is present, or that its level exceeds a user set, predefined value.

In a second embodiment, the previously described "anti-canine COX-2 antibodies" are employed in an immunodiagnostic assay.

- 5 In one sub-embodiment of this aspect of the present invention, one can ascertain the canine COX-2 Response in a biopsy (or a macrophage or other blood cell sample), or other cell sample, or more preferably, in a sample of bodily fluid (especially, blood, serum, plasma, tears, etc.).

- 10 The anti-canine COX-2 antibodies of the present invention may thus be used in an immunoassay to assess the presence of canine COX-2. Any of a wide array of immunoassays formats may be used for this purpose (Fackrell, *Clin. Immunoassay* 8:213-219 (1985)), Yolken, *Rev. Infect. Dis.* 4:35 (1982); Collins, In: *Alternative Immunoassays*, John Wiley & Sons, NY (1985); Ngo, *et al.*, In: *Enzyme Mediated Immunoassay*, Plenum Press, NY (1985)).

- 15 The simplest immunoassay involves merely incubating an antibody that is capable of binding to a predetermined target molecule with a sample suspected to contain the target molecule. The presence of the target molecule is determined by the presence, and proportional to the concentration, of any antibody bound to the target molecule. In order to facilitate the separation of target-bound antibody from the unbound antibody initially present, a solid phase is typically employed. Thus, for
20 example the sample can be passively bound to a solid support, and, after incubation with the antibody, the support can be washed to remove any unbound antibody.

- 25 In more sophisticated immunoassays, the concentration of the target molecule is determined by binding the antibody to a support, and then permitting the support to be in contact with a sample suspected of containing the target molecule. Target molecules that have become bound to the immobilized antibody can be detected in any of a variety of ways. For example, the support can be incubated in the presence of a labeled, second antibody that is capable of binding to a second epitope of the target

molecule. Immobilization of the labeled antibody on the support thus requires the presence of the target, and is proportional to the concentration of the target in the sample. In an alternative assay, the target is incubated with the sample and with a known amount of labeled target. The presence of target molecule in the sample
5 competes with the labeled target molecules for antibody binding sites. Thus, the amount of labeled target molecules that are able to bind the antibody is inversely proportional to the concentration of target molecule in the sample.

In general, immunoassay formats employ either radioactive labels ("RIAs") or enzyme labels ("ELISAs"). RIAs have the advantages of simplicity, sensitivity, and ease of use. Radioactive labels are of relatively small atomic dimension, and do not
10 normally affect reaction kinetics. Such assays suffer, however, from the disadvantages that, due to radioisotopic decay, the reagents have a short shelf-life, require special handling and disposal, and entail the use of complex and expensive analytical equipment. RIAs are described in *Laboratory Techniques and*
15 *Biochemistry in Molecular Biology*, by Work, *et al.*, North Holland Publishing Company, NY (1978), with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard. ELISAs have the advantage that they can be conducted using inexpensive equipment, and with a myriad of different enzymes, such that a large number of detection strategies --
20 colorimetric, pH, gas evolution, etc. -- can be used to quantitate the assay. In addition, the enzyme reagents have relatively long shelf-lives, and lack the risk of radiation contamination that attends to RIA use. ELISAs are described in *ELISA and Other Solid Phase Immunoassays* (Kemeny, *et al.*, Eds.), John Wiley & Sons, NY (1988).

25 Anti-canine COX-2 antibodies or canine COX-2 binding molecules may be administered to a dog, and their capacity to bind to canine COX-2 *in vivo* may be determined by ocular examination. Significantly, since such a diagnostic test is relatively rapid, immune responses that require significant time, such as the potential

eliciting of anti-[anti-canine COX-2] antibodies, or the complexing of such antibodies with anti-canine COX-2 antibodies, is not important. In a preferred embodiment, the antibody will be fluorescently labeled, and will be provided to a dog by injection into the dog's circulatory system.

- 5 In another aspect of the present invention, a canine COX-2 protein or fragment thereof can be used in assays for screening test substances for the ability to modulate or maintain canine COX-2 activity. In a sub-embodiment, the test substance is an agonist, antagonist, or small molecule inhibitor of the canine COX-2 protein. In another sub-embodiment, the test substance may bind to canine COX-2 substrate.
- 10 The test substance may also be an agonist, antagonist, or small molecule inhibitor of COX-1.

- Assays for screening canine COX-2 activity include, for example, a colorimetric assay (Kaufman et al., *Enzyme* 12:537 (1971), Richards and Greengard, *Biochimica et Biophysica Acta* 304:842-850 (1973), McKnight et al., *J. Biochem.* 267:25208-25212 (1992)) and a HPLC-based separation technique (Buse et al. *Am J. Physiol.* 272: E1080-1088 (1997), Nelson et al., *Am. J. Physiol.* 272: E848-855 (1997)).
- 15

- Accordingly, the present invention provides a transformed cell having a nucleic acid molecule which comprises an exogenous promoter region linked to a structural nucleic acid sequence, and a 3' non-translated sequence linked to the structural nucleic acid sequence, wherein the exogenous promoter region causes the production of a mRNA molecule; the structural nucleic acid sequence encodes a canine COX-1 gene or a fragment thereof; and the 3' non-translated sequence causes termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule. In one embodiment, the structural nucleic acid sequence is in the sense orientation. In an alternative embodiment, the structural nucleic acid sequence is in the antisense orientation.
- 20
- 25

In another embodiment the present invention provides a transformed cell having a nucleic acid molecule which comprises an exogenous promoter region linked to a structural nucleic acid sequence, and a 3' non-translated sequence, wherein the exogenous promoter region causes the production of a mRNA molecule; the structural
5 nucleic acid molecule encodes a canine COX-2 gene or a fragment thereof; and the 3' non-translated sequence causes termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule. In one embodiment, the structural nucleic acid sequence is in the sense orientation. In an alternative embodiment, the structural nucleic acid sequence is in the antisense
10 orientation.

In another embodiment the present invention provides a method for assaying a canine COX-2 protein in a cell comprising (A) incubating a marker nucleic acid molecule with a complementary nucleic acid molecule obtained from the cell, wherein the incubation is performed under conditions permitting nucleic acid hybridization,
15 and the marker nucleic acid molecule is capable of specifically hybridizing under high stringency conditions to a canine COX-2-encoding nucleic acid molecule or a complement thereof; (B) hybridizing the marker nucleic acid molecule with the complementary nucleic acid molecule; and (C) assaying the complementary nucleic acid, wherein the assay is predictive of the level or pattern of the canine COX-2
20 protein. In one embodiment, the assay is predictive of the canine COX-2 protein concentration. In another embodiment, the assay is predictive of the canine COX-2 protein pattern. The assay may be performed, for example, by *in situ* hybridization.

In another embodiment the present invention provides a method for diagnosis or prognosis of a COX-2 related condition in a dog which comprises assaying the
25 concentration of a molecule, the concentration being dependent upon the expression of a canine COX-2 gene, and the molecule being assayable in a biological sample from the dog. The biological sample can be practically any biological sample from the animal, for example, skeletal tissue, blood, lymph, or serum. The assayed molecule can, for example, be a protein molecule expressed by the canine COX-2
30 gene. Alternatively, the assayed molecule can be a nucleic acid molecule such as a mRNA molecule encoded by the canine COX-2 gene, or a cDNA molecule encoded

by the canine COX-2 gene. In one embodiment, the concentration of the mRNA molecule is assayed by incubating the biological sample in the presence of a nucleic acid molecule that hybridizes to the mRNA molecule to produce a hybridized mRNA molecule, and detecting the hybridized mRNA molecule.

- 5 In another embodiment the present invention provides a method for diagnosing a COX-2 related condition in a dog which comprises the steps of: (A) incubating under conditions permitting a nucleic acid hybridization a marker nucleic acid molecule with a complementary nucleic acid molecule obtained from a biological sample from the dog; (B) hybridizing the marker nucleic acid molecule with the
- 10 complementary nucleic acid molecule; and (C) assaying the hybridized marker nucleic acid molecule and complementary nucleic acid molecule for a polymorphism; thereby diagnosing the COX-2 related condition in the dog wherein: the marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that specifically hybridizes to another polynucleotide that is linked to a canine COX-2 gene; and the
- 15 polymorphism is predictive of a mutation affecting the canine COX-2 condition in the dog. Preferably, the assay detects the concentration of the hybridized marker nucleic acid molecule and complementary nucleic acid molecule. Alternatively, the assay can detect the pattern of the hybridized marker nucleic acid molecule and complementary nucleic acid molecule. In one embodiment, the marker nucleic acid molecule is a
- 20 mRNA molecule encoded by the gene or a cDNA molecule encoded by the gene. The COX-2 related condition can be any condition in which the canine COX-2 protein is expressed in the animal. For example the COX-2 related condition can be inflammation, arthritis, cancer, neoplasia, a central nervous system ("CNS") disorder, an ophthalmological disorder, bronchitis, tendinitis, bursitis, skin related conditions
- 25 (such as psoriasis, eczema, burns, or dermatitis), inflammatory bowel disease, gastritis, irritable bowel syndrome, Chron's disease, ulcerative colitis, vascular diseases, migraine headaches, periarteritis nodosa, thyroiditis, aplastic anemia, pancreatitis, Hidgkin's disease, sclerodoma, rheumatic fever, diabetes, myasthenia gravis, sarcoidosis, nephrotic syndrome, hypertensivity, conjunctivitis, gingivitis,
- 30 swelling after surgery, myocardial ischemia, and the like.

In another embodiment the present invention provides a method of determining an association between a polymorphism and a trait expressed by a dog wherein the method comprises: (A) hybridizing a nucleic acid molecule specific for the polymorphism to a gene in a cell, wherein the nucleic acid molecule has a nucleic acid sequence of SEQ ID: 3 or a complement thereof; and (B) determining the degree of association between the polymorphism and the trait. The marker nucleic acid molecule can for example comprise a nucleotide sequence of a polynucleotide that is physically linked to within 1 mb of a sequence that specifically hybridizes to the gene. The marker nucleic acid molecule can, if desired, comprise a nucleotide sequence of a polynucleotide that is physically linked to within 100 kb to a sequence that specifically hybridizes to the gene. Alternatively, the marker molecule can comprise a nucleotide sequence of a polynucleotide that is physically linked to within 10 kb to a sequence that specifically hybridizes to the gene.

In another embodiment the present invention provides a method of producing a cell containing an expressed canine COX-2 protein comprising: (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed cell; thereby producing the cell containing the expressed canine COX-2 protein, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region, the structural region comprises a nucleic acid sequence of SEQ ID: 3, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and the functional nucleic acid molecule results in expression of the canine COX-2 protein.

In another embodiment the present invention provides a method of producing a cell exhibiting co-suppression of a canine COX-2 protein comprising: (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed cell; thereby producing the cell exhibiting co-suppression of the canine COX-2 protein, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region, the structural region comprises a nucleic acid sequence of SEQ ID: 3, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription

and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in co-suppression of the canine COX-2 protein.

In another embodiment the present invention provides a method for causing
5 co-suppression of a canine COX-2 protein in a transformed cell comprising: (A) transforming the cell with a nucleic acid molecule to produce a transformed cell; and (B) culturing the transformed cell; thereby causing co-suppression of the canine COX-2 protein in the transformed cell, wherein: the functional nucleic acid molecule comprises a promoter region; the promoter region is linked to a structural region, the
10 structural region comprises a nucleic acid molecule having a nucleic acid sequence of SEQ ID: 3, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in co-suppression of the canine COX-2 protein.

15 In another embodiment the present invention provides a method of isolating a nucleic acid that encodes a canine COX-2 protein or fragment thereof comprising (A) incubating under conditions permitting nucleic acid hybridization a first nucleic acid molecule comprising SEQ ID: 3 or the complement thereof with a complementary second nucleic acid molecule obtained from a cell; (B) hybridizing the first nucleic
20 acid molecule with the second nucleic acid molecule; and (C) isolating the second nucleic acid molecule.

In another embodiment the present invention provides a method of producing a cell containing an expressed canine COX-2 gene comprising (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed cell;
25 thereby producing the cell containing the expressed canine COX-2 gene, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region wherein the structural region comprises a nucleic acid molecule having a nucleic acid sequence consisting of SEQ ID:3 or complements thereof the structural region is linked to a 3' non-translated sequence that functions in
30 the cell to cause termination of transcription and addition of polyadenylated

ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in overexpression of the canine COX-2 gene protein.

In another embodiment the present invention provides a method of producing a cell exhibiting co-suppression of a canine COX-2 gene comprising (A) transforming
5 the cell with a functional nucleic acid molecule; and (B) culturing the transformed cell; thereby producing a cell co-suppression of a canine COX-2 gene, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region wherein the structural region comprises a nucleic acid sequence selected from the group consisting of SEQ ID: 3 or complements thereof,
10 the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in co-suppression of the canine COX-2 gene.

In another embodiment the present invention provides a method for causing
15 co-suppression of a canine COX-2 gene in a cell comprising (A) transforming the cell with a nucleic acid molecule; and (B) culturing the transformed cell; thereby reducing expression of a canine COX-2 gene in a cell, wherein the nucleic acid molecule has an exogenous promoter region which causes the production of a mRNA molecule, the exogenous promoter region is linked to a transcribed nucleic acid molecule having a
20 transcribed strand and a non-transcribed strand, the transcribed strand is complementary to a nucleic acid molecule having a nucleic acid selected from the group consisting of SEQ ID: 3 or complements thereof and the transcribed strand is complementary to an endogenous mRNA molecule, and the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the cell to cause
25 termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule.

In another embodiment the present invention provides a substantially-purified nucleic acid molecule comprising a sequence which encodes a mammalian canine COX-2 protein. Preferably the substantially-purified nucleic acid encodes a sequence
30 selected from the group consisting of human, rat, or mouse canine COX-2 protein.

More preferably it encodes a human canine COX-2 protein. More preferably still it encodes a mouse canine COX-2.

In another embodiment the present invention provides a method of identifying a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises (A) contacting a test transformed cell with the test material, wherein the transformed cell expresses a gene encoding canine COX-2, and wherein the contacting is performed in the presence of test amount of arachadonic acid; (B) measuring the conversion of prostaglandin precursor to prostaglandin product by the test transformed cells; (C) contacting a control transformed cell with a control amount of prostaglandin precursor, wherein the control transformed cell expresses a gene encoding canine COX-2, and wherein the contacting is performed substantially in the absence of the test material; (D) measuring the conversion of prostaglandin precursor to prostaglandin product by the control transformed cells; and (E) comparing the amount of prostaglandin precursor converted to prostaglandin product by the test transformed cells to the amount of prostaglandin precursor converted to prostaglandin product by the control transformed cells.

In another embodiment the present invention provides a method of identifying a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises (A) contacting a test canine COX-2 protein with the test material, wherein the contacting is performed in the presence of test amount of a prostaglandin precursor; (B) measuring the conversion of the prostaglandin precursor to a prostaglandin product by the test transformed cells; (C) contacting a control canine COX-2 protein with a control amount of the prostaglandin precursor, wherein the contacting is performed substantially in the absence of the test material; (D) measuring the conversion of the prostaglandin precursor to the prostaglandin product by the canine COX-2 protein; and (E) comparing the amount of

the prostaglandin precursor converted to prostaglandin product by the test canine COX-2 protein to the amount of prostaglandin precursor converted to prostaglandin product by the control canine COX-2 protein.

In another embodiment the present invention provides a method of identifying
5 a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises (A) contacting a test canine COX-2 fragment with the test material, wherein the contacting is performed in the presence of test amount of a prostaglandin precursor; (B) measuring the conversion of the prostaglandin precursor to a prostaglandin product by the test transformed cells; (C)
10 contacting a control canine COX-2 amino acid fragment with a control amount of the prostaglandin precursor, wherein the contacting is performed substantially in the absence of the test material; (D) measuring the conversion of the prostaglandin precursor to the prostaglandin product by the canine COX-2 amino acid fragment; and (E) comparing the amount of the prostaglandin precursor converted to prostaglandin
15 product by the test canine COX-2 amino acid fragment to the amount of prostaglandin precursor converted to prostaglandin product by the control canine COX-2 amino acid fragment.

In a preferred embodiment, the prostaglandin precursor is arachadonic acid.

20 Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Example 1

25 cDNA Isolation

For construction of cDNA libraries for cloning canine COX-1 and COX-2, mRNA was prepared from canine kidney papillae and lipopolysaccharide (LPS)-stimulated canine mononuclear cells.

For the kidney cDNA library construction, total RNA was isolated from 200
5 micrograms (ug) of kidney tissue using 12 ml of TRIzol reagent (Life Technologies). RNA was prepared using the standard protocol provided by the manufacturer (Life Technologies). The protocol yielded about 200 ug of total RNA. The mRNA was then isolated using a Mini Oligo(dT) Cellulose Spin Column Kit (5 prime-3 prime). The yield was about 7 ug after a single oligo(dT) column purification.

10 For construction of a mononuclear cell cDNA library, mRNA was isolated directly from about 10^7 cells using the Poly(A) Pure mRNA Purification Kit (Ambion). After a single oligo(dT) purification step, 2 ug of mRNA were isolated.

Example 2

Gene Expression

15 Canine COX-1 was expressed using a full-length COX-1 cDNA containing the entire coding region, which was digested with Sall and NotI to generate ca.2600 base pair fragment for ligation into an insect cell expression vector, pFastBac 1 (Invitrogen), which had been digested with Sall and NotI. The resulting plasmid, pMON37775, was sequenced to confirm cloning.

20 An insect cell expression plasmid for canine COX-2 was created by ligating a COX-2 fragment generated by PCR fragment to a partial COX-2 cDNA. The 5' end of COX-2 was cloned from kidney double-stranded cDNA using PCR. Both fragments were ligated into pFastBack 1, which had been digested with EcoRI and NotI. The resulting plasmid, pMON37776, was confirmed by sequencing.

Example 3

Selective COX-2 Inhibitor Screening Assay

The standard screening assay for inhibitor potency (IC₅₀) was an ELISA method for detection of PGE₂. IC₅₀ values were determined by pre-incubation of the inhibitors with enzyme for 10 min. at 25 C in a buffer containing 50 mM potassium phosphate, pH 7.5, 2 mM phenol, 1 mM heme, and 300 mM epinephrine. Inhibitors
5 were first made as a 10 mM stock in DMSO and further diluted into 1:9 (DMSO:ethanol). The reaction was allowed to proceed for 10 min. For time dependent inhibition, the initial rate of the reaction was measured by stopping the reaction at 15 sec. with the addition of 1/10 volume N-HCl; the amount of prostaglandin formed was measured by ELISA (Cayman Chemical Co., Ann Arbor,
10 MI). IC₅₀s were determined from a 4 parameter log fit of the data using Grafit.

All references, patents or applications cited herein are incorporated by reference in their entirety as if written herein.

We claim:

1. A substantially-purified nucleic acid molecule selected from the group of DNAs consisting of a nucleic acid molecule having SEQ ID: 1, the
5 complement of the nucleic acid molecule having SEQ ID:1, an internal fragment of the nucleic acid molecule having SEQ ID: 1, and an internal fragment of the complement of the nucleic acid molecule having SEQ ID: 1, wherein SEQ ID: 1 encodes a canine COX-1 protein or a fragment thereof.
- 10 2. The substantially-purified nucleic acid molecule according to claim 1, wherein the substantially-purified nucleic acid molecule encodes the canine COX-1 protein.
3. The substantially-purified nucleic acid molecule according to claim 1, wherein
15 the substantially-purified nucleic acid molecule has SEQ ID: 1 or a complement thereof.
4. The substantially-purified nucleic acid molecule according to claim 1, wherein
20 the nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-1 or complement thereof and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-1 or complement thereof.
5. The substantially-purified nucleic acid molecule according to claim 4, wherein
25 the nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-1 or complement thereof under high stringency conditions and fails to specifically hybridize to a nucleic acid

molecule that encodes non-canine COX-1 or complement thereof under high stringency conditions.

6. A substantially-purified canine COX-1 nucleic acid molecule which comprises a nucleic acid sequence that is identical to at least 20 contiguous nucleotides of SEQ ID: 1 or a complement thereof.
7. The substantially-purified canine COX-1 nucleic acid molecule according to claim 6, wherein the nucleic acid molecule comprises a nucleic acid sequence that is identical to at least 50 contiguous nucleotides of SEQ ID: 1 or a complement thereof.
8. The substantially-purified canine COX-1 nucleic acid molecule according to claim 7, wherein the nucleic acid molecule comprises a nucleic acid sequence that is identical to at least 100 contiguous nucleotides of SEQ ID: 1 or a complement thereof.
9. A substantially-purified canine COX-1 protein or a fragment thereof wherein the canine COX-1 protein or the fragment is encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 1 or a complement thereof.
10. A substantially-purified canine COX-1 protein or a fragment thereof according to claim 9, where the nucleic acid sequence is selected from SEQ ID: 1 or a complement thereof.
11. A substantially-purified canine COX-1 protein having SEQ ID: 2.
12. A substantially-purified canine COX-1 protein or a fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 2.

- 13 The substantially-purified canine COX-1 protein or a fragment thereof
according to claim 12, wherein the canine COX-1 protein or fragment is a
fusion protein.
14. An antibody capable of specifically binding to a substantially-purified canine
5 COX-1 protein having SEQ ID: 2.
15. The antibody of claim 14, wherein the antibody is detectably labeled.
16. A substantially-purified nucleic acid molecule selected from the group of
DNAs consisting of a nucleic acid molecule having SEQ ID: 3, a complement
molecule of a nucleic acid molecule having SEQ ID: 3, an internal fragment a
10 nucleic acid molecule having SEQ ID: 3, and an internal fragment of a
complement molecule of a nucleic acid molecule having SEQ ID: 3, wherein
the SEQ ID: 3 encodes a canine COX-2 protein or a fragment thereof.
17. The substantially-purified nucleic acid molecule according to claim 16,
wherein the substantially-purified nucleic acid molecule encodes the canine
15 COX-2 protein.
18. The substantially-purified nucleic acid molecule according to claim 16,
wherein the substantially-purified nucleic acid molecule has the sequence
described by SEQ ID: 3 or a complement thereof.
- 20 19. The substantially-purified nucleic acid molecule according to claim 16,
wherein the substantially-purified nucleic acid molecule specifically
hybridizes to another nucleic acid molecule that encodes canine COX-2 or
complement thereof and fails to specifically hybridize to a nucleic acid
25 molecule that encodes non-canine COX-2 or complement thereof.

20. The substantially-purified nucleic acid molecule according to claim 19,
wherein the substantially-purified nucleic acid molecule specifically
hybridizes to another nucleic acid molecule that encodes canine COX-2 or
complement thereof under high stringency conditions and fails to specifically
hybridize to a nucleic acid molecule that encodes non-canine COX-2 or
complement thereof under high stringency conditions.
21. A substantially-purified canine COX-2 nucleic acid molecule which comprises
a nucleic acid sequence that is identical to at least 20 contiguous nucleotides of
SEQ ID: 3 or a complement thereof.
22. The substantially-purified canine COX-2 nucleic acid molecule according to
claim 21, wherein the nucleic acid molecule comprises a nucleic acid sequence
that is identical to at least 50 contiguous nucleotides of SEQ ID: 3 or a
complement thereof.
23. The substantially-purified canine COX-2 nucleic acid molecule according to
claim 22, wherein the nucleic acid molecule comprises a nucleic acid sequence
that is identical to at least 100 contiguous nucleotides of SEQ ID: 3 or its
complement.
24. A substantially-purified canine COX-2 protein or a fragment thereof encoded
by a nucleic acid sequence which specifically hybridizes to SEQ ID: 4 or its
complement.
25. The substantially-purified canine COX-2 protein or a fragment thereof
according to claim 24, where the nucleic acid sequence is selected from the
group consisting of SEQ ID: 4 and the complement of SEQ ID: 4.
26. A substantially-purified canine COX-2 protein having SEQ ID: 4.

27. A substantially-purified canine COX-2 protein or a fragment thereof wherein the substantially-purified canine COX-2 protein or the fragment thereof comprises at least 10 consecutive amino acids of SEQ ID: 4.
28. The substantially-purified canine COX-2 protein or fragment thereof
5 according to claim 27, wherein the canine COX-2 protein or fragment thereof is a fusion protein.
29. An antibody capable of specifically binding to the substantially-purified canine COX-2 protein having SEQ ID: 4.
30. The antibody of claim 29, wherein the antibody is detectably labeled.
- 10 31. A transformed cell having a nucleic acid molecule which comprises an exogenous promoter region linked to a structural nucleic acid sequence, and a 3'non-translated sequence linked to the structural nucleic acid sequence, wherein:
- the exogenous promoter region causes production of a mRNA
15 molecule;
- the structural nucleic acid sequence encodes a canine COX-1 gene or a fragment thereof; and
- the 3' non-translated sequence causes termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.
- 20 32. The transformed cell according to claim 31, wherein the structural nucleic acid sequence is in the antisense orientation.
33. A transformed cell having a nucleic acid molecule which comprises an exogenous promoter region linked to a structural nucleic acid sequence, and a
25 a 3' non-translated sequence, wherein:

the exogenous promoter region causes production of a mRNA molecule;

the structural nucleic acid molecule encodes a canine COX-2 gene or a fragment thereof; and

5 the 3' non-translated sequence causes termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

34. The transformed cell according to claim 33, wherein the structural nucleic acid sequence is in the antisense orientation.

35. A method for assaying a canine COX-2 protein in a cell comprising:

10 (A) incubating a marker nucleic acid molecule with a complementary nucleic acid molecule obtained from the cell, wherein:
the incubation is performed under conditions permitting nucleic acid hybridization; and
the marker nucleic acid molecule is capable of specifically hybridizing
15 under high stringency conditions to a canine COX-2-encoding nucleic acid molecule or a complement thereof;

(B) hybridizing the marker nucleic acid molecule with the complementary nucleic acid molecule; and

(C) assaying the complementary nucleic acid, wherein the assay is
20 predictive of the level or pattern of the canine COX-2 protein.

36. The method of claim 35, wherein the assay is predictive of the canine COX-2 protein concentration.

37. The method of claim 35, wherein the assay is predictive of the canine COX-2 protein pattern.

25 38. The method of claim 35, wherein the assay is performed by *in situ* hybridization.

39. A method for diagnosis or prognosis of a COX-2 related condition in a dog wherein the method comprises assaying the concentration of a molecule, the concentration being dependent upon the expression of a canine COX-2 gene, and the molecule being assayable in a biological sample from the dog.
- 5 40. The method of claim 39, wherein the biological sample is selected from the group consisting of skeletal tissue, blood, lymph and serum.
41. The method of claim 39, wherein the molecule is a protein molecule expressed by the canine COX-2 gene.
42. The method of claim 39, wherein the molecule is a mRNA molecule encoded
10 by the canine COX-2 gene, or a cDNA molecule encoded by the canine COX-2 gene.
43. The method of claim 42, wherein the concentration of the mRNA molecule is assayed by incubating the biological sample in the presence of a nucleic acid molecule that hybridizes to the mRNA molecule to produce a hybridized
15 mRNA molecule, and detecting the hybridized mRNA molecule.
44. A method for diagnosing a COX-2 related condition in a dog which comprises the steps of:
- (A) incubating under conditions permitting a nucleic acid hybridization a
marker nucleic acid molecule with a complementary nucleic acid
20 molecule obtained from a biological sample from the dog;
- (B) hybridizing the marker nucleic acid molecule with the complementary nucleic acid molecule; and
- (C) assaying the hybridized marker nucleic acid molecule and complementary nucleic acid molecule for a polymorphism;

thereby diagnosing the COX-2 related condition in the dog, wherein:

the marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that specifically hybridizes to another polynucleotide that is linked to a canine COX-2 gene; and

5 the polymorphism is predictive of a mutation affecting the canine COX-2 condition in the dog.

45. The method of claim 44, wherein the assay detects the concentration of the hybridized marker nucleic acid molecule and complementary nucleic acid molecule .
- 10 46. The method of claim 44, wherein the assay detects the pattern of the hybridized marker nucleic acid molecule and complementary nucleic acid molecule .
47. The method of claim 44, wherein the marker nucleic acid molecule is a mRNA molecule encoded by the canine COX-2 gene or a cDNA molecule encoded by the canine COX-2 gene.
- 15 48. The method of claim 44, wherein the COX-2 related condition is inflammation.
49. The method of claim 44, wherein the COX-2 related condition is arthritis.
50. The method of claim 44, wherein the COX-2 related condition is cancer.
- 20 51. The method of claim 44, wherein the COX-2 related condition is neoplasia.
52. The method of claim 44, wherein the COX-2 related condition is a CNS disorder.

53. A method of determining an association between a polymorphism and a trait wherein the method comprises:
- (A) hybridizing a nucleic acid molecule specific for the polymorphism to a gene in a cell, wherein the nucleic acid molecule has a nucleic acid sequence of SEQ ID: 3 or a complement thereof; and
- (B) determining the degree of association between the polymorphism and the trait.
54. The method of claim 53, wherein the marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is physically linked to within 1 mb of a sequence that specifically hybridizes to the gene.
55. The method of claim 53, wherein the marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is physically linked to within 100 kb to a sequence that specifically hybridizes to the gene.
56. The method of claim 53, wherein the marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is physically linked to within 10 kb to a sequence that specifically hybridizes to the gene.
57. A method of producing a cell containing an expressed canine COX-2 protein comprising:
- (A) transforming the cell with a functional nucleic acid molecule; and
- (B) culturing the transformed cell;
- thereby producing the cell containing the expressed canine COX-2 protein, wherein:
- the functional nucleic acid molecule comprises a promoter region;
- the promoter region is linked to a structural region;
- the structural region comprises a nucleic acid sequence of SEQ ID: 3,

the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and

the functional nucleic acid molecule results in expression of the canine

5 COX-2 protein.

58. A method of producing a cell exhibiting co-suppression of a canine COX-2 protein comprising:

(A) transforming the cell with a functional nucleic acid molecule; and

(B) culturing the transformed cell;

10 thereby producing the cell exhibiting co-suppression of the canine COX-2 protein, wherein:

the functional nucleic acid molecule comprises a promoter region;

the promoter region is linked to a structural region;

the structural region comprises a nucleic acid sequence of SEQ ID: 3;

15 the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and

the functional nucleic acid molecule results in co-suppression of the canine COX-2 protein.

20 59. A method for causing co-suppression of a canine COX-2 protein in a transformed cell comprising:

(A) transforming the cell with a nucleic acid molecule to produce a transformed cell; and

(B) culturing the transformed cell;

25 thereby causing co-suppression of the canine COX-2 protein in the transformed cell, wherein:

the functional nucleic acid molecule comprises a promoter region;

the promoter region is linked to a structural region;

the structural region comprises a nucleic acid molecule having a nucleic acid sequence of SEQ ID: 3;

the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and

the functional nucleic acid molecule results in co-suppression of the canine COX-2 protein.

60. A method of isolating a nucleic acid molecule that encodes a canine COX-2 protein or a fragment thereof comprising:

- (A) incubating under conditions permitting nucleic acid hybridization a first nucleic acid molecule comprising SEQ ID: 3 or the complement thereof with a complementary second nucleic acid molecule obtained from a cell;
- (B) hybridizing the first nucleic acid molecule with the second nucleic acid molecule; and
- (C) isolating the second nucleic acid molecule.

61. A method of producing a cell containing an expressed canine COX-2 gene comprising:

- (A) transforming the cell with a functional nucleic acid molecule; and
- (B) culturing the transformed cell.

thereby producing the cell containing the expressed canine COX-2 gene, wherein:

the functional nucleic acid molecule comprises a promoter region;

5 the promoter region is linked to a structural region wherein the structural region comprises a nucleic acid molecule having a nucleic acid sequence consisting of SEQ ID:3 or complements thereof;

the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and

10 the functional nucleic acid molecule results in overexpression of the canine COX-2 gene protein.

62. A method of producing a cell exhibiting co-suppression of a canine COX-2 gene comprising:

(A) transforming the cell with a functional nucleic acid molecule; and

15 (B) culturing the transformed cell;

thereby producing a cell co-suppression of a canine COX-2 gene, wherein:

the functional nucleic acid molecule comprises a promoter region;

20 the promoter region is linked to a structural region wherein the structural region comprises a nucleic acid sequence selected from the group consisting of SEQ ID: 3 or complements thereof;

the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and

the functional nucleic acid molecule results in co-suppression of the canine COX-2 gene.

63. A method for causing co-suppression of a canine COX-2 gene in a cell comprising:

- (A) transforming the cell with a nucleic acid molecule; and
- (B) culturing the transformed cell;

thereby reducing expression of a canine COX-2 gene in a cell, wherein:

the nucleic acid molecule has an exogenous promoter region which functions in a cell to cause the production of a mRNA molecule;

the exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand;

the transcribed strand is complementary to a nucleic acid molecule having a nucleic acid selected from the group consisting of SEQ ID: 3 or complements thereof and the transcribed strand is complementary to an endogenous mRNA molecule; and

the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule.

64. A method of identifying a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises:

- 5 (A) contacting a test transformed cell with the test material, wherein the test transformed cell expresses a gene encoding canine COX-2, and wherein the contacting is performed in the presence of test amount of a prostaglandin precursor;
- (B) measuring the conversion of prostaglandin precursor to a prostaglandin product by the test transformed cell;
- 10 (C) contacting a control transformed cell with a control amount of the prostaglandin precursor, wherein the control transformed cell expresses a gene encoding canine COX-2, and wherein the contacting is performed substantially in the absence of the test material;
- (D) measuring the conversion of the prostaglandin precursor to
15 prostaglandin product by the control transformed cell; and
- (E) comparing the amount of the prostaglandin precursor converted to prostaglandin product by the test transformed cell to the amount of prostaglandin precursor converted to prostaglandin product by the control transformed cell, thereby identifying the test material.

20 65. A method of identifying a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises:

- (A) contacting a canine COX-2 protein with the test material, and wherein the contacting is performed in the presence of test amount of a prostaglandin precursor;
- (B) measuring the conversion of the prostaglandin precursor to a prostaglandin product;
- (C) contacting a control canine COX-2 protein with a control amount of the prostaglandin precursor, and wherein the contacting is performed substantially in the absence of the test material;
- (D) measuring the conversion of the prostaglandin precursor to prostaglandin product; and
- (E) comparing the amount of the prostaglandin precursor converted to prostaglandin product by the canine COX-2 protein to the amount of the prostaglandin precursor converted to prostaglandin product by the control canine COX-2 protein, thereby identifying the test material.
66. A method of identifying a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises:
- (A) contacting a canine COX-2 amino acid fragment with the test material, and wherein the contacting is performed in the presence of test amount of a prostaglandin precursor;
- (B) measuring the conversion of the prostaglandin precursor to a prostaglandin product;

(C) contacting a control canine COX-2 amino acid fragment with a control amount of the prostaglandin precursor, and wherein the contacting is performed substantially in the absence of the test material;

(D) measuring the conversion of the prostaglandin precursor to prostaglandin product; and

(E) comparing the amount of the prostaglandin precursor converted to prostaglandin product by the canine COX-2 amino acid fragment to the amount of the prostaglandin precursor converted to prostaglandin product by the control canine COX-2 amino acid fragment, thereby identifying the test material.

67. The method of claims 64, 65, or 66 wherein the prostaglandin precursor is arachadonic acid.

1/10

CANINE MSRGSR LHRW PLLL--LL-L PLLLPPPVLP AE--ARTPAP VNPCCYYP CQ
 ::: : ::: :: : ::::: :::: :. : : : :::::
 HUMAN MSR-S----- -LLLRFLFL LLLPPLPVLL ADPGAPT--P VNPCCYYP CQ 41

HQGICVREGL DRYQCDCTRT GYSGPNCTIP ELWTWLRNSL RSPSFLHFL
 ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 HQGICVREGL DRYQCDCTRT GYSGPNCTIP GLWTWLRNSL RSPSFLHFL 91

LTHGRWFWEF INATFIRDML MRLVLTARSN LIPSPPTYNI AHDIYSWESF
 ::::: .::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 LTHGRWFWEF VNATFIREMR MRLVLTARSN LIPSPPTYNS AHDIYSWESF 141

SNVSYTRVL PSVPQDCPTP MGTKGKKQLP DAQLLGRRFL LRRKFIPDPQ
 ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 SNVSYTRIL PSVPKDCPTP MGTKGKKQLP DAQLLARFL LRRKFIPDPQ 191

GTNLMFAFFA QHFTHQFFKT SGKMGPGFTK DAQLLGRRFL IYGNLDRQY
 ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 GTNLMFAFFA QHFTHQFFKT SGKMGPGFTK DAQLLARFL IYGNLERQY 241

QLRLFKDGKL KYQVLDGEMY PPSVEEAPVL MHYPRGILPQ SQMAVGQEVF
 ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 QLRLFKDGKL KYQVLDGEMY PPSVEEAPVL MHYPRGIPPQ SQMAVGQEVF 250

GLLPGLMLYA TLWLREHNRV CDLLKAEHPT WDGEQLFQTA RLILIGETIK
 ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 GLLPGLMLYA TLWLREHNRV CDLLKAEHPT WDGEQLFQTT RLILIGETIK 300

IVIEEYVQQL SGYFLQLKFD PELLFSAQFQ YRNRIAMEFN QLYHWHPLMP
 ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 IVIEEYVQQL SGYFLQLKFD PELLFGVQFQ YRNRIAMEFN HLYHWHPLMP 350

DSFWVGSQEY SYEQFLENTS MLTHYGIEAL VDAFSRQSAG RIGGGRNIDH
 ::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 DSFKVGSQEY SYEQFLENTS MLVDYGVEAL VDAFSRQIAG RIGGGRNMDH 400

fig. 1A

3/10

CANINE MLFRAVLLCA ALAVVRAANP CSHPCQNOG ICMSTGFDQY KCDCTRTGFI
 :: ::::: ::. :::: ::::: : :::: ::::: ::::: :::::
 HUMAN MLARALLCA VLALSHTANP CSHPCQNRG VCMVGFDQY KCDCTRTGFI 50

GENCSTPEFL TRIKLYLKPT PNTVHYILTH FKGWNVNIN IPFLRNTIMK
 ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 GENCSTPEFL TRIKFLKPT PNTVHYILTH FKGWNVNIN IPFLRNAIMS 100

YVLTSRSHLI ESPPTYNVNY GYKSWEAFSN LSYTRALPP VPDDCPTMG
 ::::: ::::: : ::::: ::::: ::::: ::::: :::::
 YVLTSRSHLI DSPPTYNADY GYKSWEAFSN LSYTRALPP VPDDCPTPLG 150

VKGKKELPDS KEIVEKFLR RKFIPDQGT NMFAPFAQH FTHQFFKTDH
 ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 VKGKKQLPDS NEIVGKLLR RKFIPDQGS NMFAPFAQH FTHQFFKTDH 200

KRGPAFTKGL GHGVDLNHVI GETLDRQHL RLFKDGKMY QVIDGEVYPP
 ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 KRGPAFTNGL GHGVDLNHTY GETLARQKL RLFKDGKMY QIIDGEMYPP 250

TVKDTQVEMI YPPHVPEHLQ FAVGQEVFGL VPGLMMYATI WLREHNRVCD
 ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 TVKDTQAEMI YPPQVPEHLR FAVGQEVFGL VPGLMMYATI WLREHNRVCD 300

VLKQEHPEWG DEQLFQTSRL ILIGETIKIV IEDYVQHL SG YHFKLKFDPE
 ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 VLKQEHPEWG DEQLFQTSRL ILIGETIKIV IEDYVQHL SG YHFKLKFDPE 350

LLFNQQFQYQ NRIAAEFNTL YHWHPLLPDT LQIDDQEYNF QQFIYNN SIL
 ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 LLFNKQFQYQ NRIAAEFNTL YHWHPLLPDT FQIDHQKYNF QQFIYNN SIL 400

LEHGLTQFVE SFTRQIAGRV AGGRNVPPAV QVSKASIDQ SRQMKYQSLN
 ::::: ::::: ::::: ::::: ::::: ::::: ::::: :::::
 LEHGITQFVE YESFEELTGE AGGRNVPPAV QVSKASIDQ SRQMKYQSFN 450

fig. 2A


```

EYKRFRFLKP YTSFEELTGE KEMAAGLEAL YGDIDAMELY PALLVEKPRP
::: :: : ::::: ::::: ::::: :::::
EYKRFRMLKP YESFEELTGE KEMSAEAL YGDIDAVELY PALLVEKPRP 500

```

```
EYRKRFRLKP MGAPFSLKGL MGNPICSPDY WKPSTFGGEV GFKIINTASI  
::: :::: : : ::::: : : :::::  
EYRKRFMLKP VGAPFSLKGL MGNVICSPAY WKPSTFGGEV GFQIINTASI 550
```

QSLICNNVKG CTFTAFSVQD GQLTKVTIN ASSSHSGLDD PDPHQDGGPG
:::~::~: : : ::::: ~::: ~::: ~::: ~:::
QSLICNNVKG CTFTSFSPVD PELIKTVTIN ASSRSGLDD PDASQDDGPA 600

STEL
:::
STEL

fig. 2B

5/10

COX2_MOUSE
COX2_RAT
COX2_GUINEAPIG
COX2_RABBIT
COX2_HORSE
COX2_CANINE
COX2_SHEEP
COX2_HUMAN
COX2_CHICKEN

MLFRAVLLCAALGLSQAANPCCSNPQNRNGECMSTGFDQYKCDCTRTGFGYGENCTTPEFL
MLFRAVLLCAALALSHAANPCCSNPQNRNGECMSIGFDQYKCDCTRTGFGYGENCTTPEIL
MLARALLCAALALGQAANPCCSNPQNRNGECLVGFDQYKCDCTRTGFGYGENCTTPEFL
MLARALLCAAVALSQAANPCCSNPQNRNGVCMTGMFDQYKCDCTRTGFGYGENCTTPEFL
MLARALLCVALALGHAANPCCSNPQNRNGVCMSTGFDQYKCDCTRTGFGYGENCTTPEFL
MLARALVLCALAVRAANPCCSNPQNRNGVCMSTGFDQYKCDCTRTGFGYGENCTTPEFL
MLARALLCAAVCG-AANPCCSNPQNRNGVCMSTGFDQYKCDCTRTGFGYGENCTTPEFL
MLARALLCAVLALSHATANPCCSNPQNRNGVCMSTGFDQYKCDCTRTGFGYGENCTTPEFL
MLLPCALLAALLAAGHAANPCCSNPQNRNGVCMSTGFDQYKCDCTRTGFGYGENCTTPEFL
** . **.. : ***** **.. ***** **.. ***** **.. ***** **..

COX2_MOUSE
COX2_RAT
COX2_GUINEAPIG
COX2_RABBIT
COX2_HORSE
COX2_CANINE
COX2_SHEEP
COX2_HUMAN
COX2_CHICKEN

TRIKLLLKPTPNTVHYILTHFKGVWNI VNNIPFLRSLTMKYVLT SRSYLIDSPPTYNVHY
TRIKLLLKPTPNTVHYILTHFKGVWNI VNNIPFLRNSIMRYVLT SRSHLIDSPPTYNVHY
TRIKLLLKPTPNTVHYILTHFKGVWNI VNNIPFLRNAIMIYVLT SRSHLIDSPPTYNAHY
TRIKLLLKPTPNTVHYILTHFKGVWNI VNSIPFLRNSIMKYVLT SRSHMIDSPPTYNVHY
TRIKLFLKPTPNTVHYILTHFKGVWNI VNSIPFLRNAVMKYVLT SRSHLIESPPTYNAQY
TRIKLYLKPTPNTVHYILTHFKGVWNI VNSIPFLRNTIMKYVLT SRSHLIESPPTYNVNY
TRIKLLLKPTPNTVHYILTHFKGVWNI VNKISFLRNMIMRYVLT SRSHLIESPPTYNVHY
TWLKLILKPTPNTVHYILTHFKGFWNI INNISFLRDTIMRYVLT SRSHLIDSPPTYNSDY
TWLKLILKPTPNTVHYILTHFKGVWNI INNISFLRDTIMRYVLT SRSHLIDSPPTYNSDY
* ** ***** **.. ***** **.. ***** **.. ***** **.. *

fig. 3A

6/10

COX2_MOUSE	GYKSWAEFNSLSYYTRALPPVADDDCPTPMGVKGKNGKELPDSKEVLEKVLRRREFIPDPQGS
COX2_RAT	GYKSWAEFNSLSYYTRALPPVADDDCPTPMGVKGKNGKELPDSKEVLEKVLRRREFIPDPQGT
COX2_GUINEAPIG	GYKSWAEFNSLSYYTRALPPVADDDCPTPMGVKGKNGKELPDSNEVLEKVLRRKFIPDPQGT
COX2_RABBIT	NYKSWAEFNSLSYYTRALPPVADDDCPTPMGVKGKNGKELPDSKDVEKLLRRKFIPDPQGT
COX2_HORSE	GYKSWESFNSLSYYTRALPPVADGCPPTPMGVKGKNGKELPDSKEIVEKFLRRKFIPDPQGT
COX2_CANINE	GYKSWESFNSLSYYTRALPPVPDDCPTPMGVKGKNGKELPDSKEIVEKFLRRKFIPDPQGT
COX2_SHEEP	SYKSWAEFNSLSYYTRALPPVPDDCPTPMGVKGKNGKELPDSKEVKKVLLRRKFIPDPQGT
COX2_HUMAN	GYKSWAEFNSLSYYTRALPPVPDDCPTPLGVKGKNGKELPDSNEIVEKLLRRKFIPDPQGS
COX2_CHICKEN	SYKSWEAAYSLSYYTRSLPPVGHDCPTPMGVKGKNGKELPDSKLIVEKFLRRKFIPDPQGT

COX2_MOUSE	NMMFAFFAQHFTHQFFKTDHKRGPGFTRGLGHGVDLNHIYGETLDRQHKLRLFKDGKCLKY
COX2_RAT	NMMFAFFAQHFTHQFFKTDQKRGPGFTRGLGHGVDLNHVYGETLDRQHKLRLFKDGKCLKY
COX2_GUINEAPIG	NMMFAFFAQHFTHQFFKSDQKRGPAFTGLAHGVDLSHIYGETLDRQHKLRLFKDGKMKY
COX2_RABBIT	NMMFAFFAQHFTHQFFKTDLKRGPFTKGLGHGVDLNHIYGETLDRQHKLRLFKDGKMKY
COX2_HORSE	NMMFAFFAQHFTHQFFKTDPKRGPAFTKGLGHGVDLSHIYGETLDRQHKLRLFKDGKMKY
COX2_CANINE	NMMFAFFAQHFTHQFFKTDHKRGPAFTKGLGHGVDLNHVYGETLDRQHKLRLFKDGKMKY
COX2_SHEEP	NLMEAFFAQHFTHQFFKTDIERGPFTKGKNGVDLSHVYGESLERQHNRRLFKDGKMKY
COX2_HUMAN	NMMFAFFAQHETHQFFKTDHKRGPAFTNGLGHGVDLNHIYGETLARQKRLRLFKDGKMKY
COX2_CHICKEN	NLMEAFFAQHFTHQFFKTDIERGPFTKGKNGVDLSHVYGETLERQHNRRLFKDGKMKY

fig. 3B

8/10

COX2_MOUSE	NRIASEFNTLYHWHPLLPDTENIEDQEYSFKQFLYNNNSILLEHGLTQFVESFTRQIAGRV
COX2_RAT	NRIASEFNTLYHWHPLLPDTENIEDQEYTFKQFLYNNNSILLEHGLAHFVESFTRQIAGRV
COX2_GUINEAPIG	NRIASEFNTLYHWHPLLPDTFQIDDDQVYNFKQFLYNNNSILLEHGLAHFVESFTRQIAGRV
COX2_RABBIT	NRIAAEFNTLYHWHPLLPDTFQIDDDQQYNYQQFLYNNNSILLEHGLTQFVESFTRQIAGRV
COX2_HORSE	NRIAAEFNTLYHWHPLLPDTFQIDDDQYNFQQFLYNNNSILVEHGLTQFVESFSRQIAGRV
COX2_CANINE	NRIAAEFNTLYHWHPLLPDTLQIDDDQYNFQQFIYNNNSILLEHGLTQFVESFSRQIAGRV
COX2_SHEEP	NRIAAEFNTLYHWHPLLPDVFQIDGQYNYQQFIYNNNSVLLHGHVTVQFVESETRQIAGRV
COX2_HUMAN	NRIAAEFNTLYHWHPLLPDTFQIHDQKYNQYQQFIYNNNSILLEHGITQFVESETRQIAGRV
COX2_CHICKEN	NRIAAEFNTLYHWHPLLPDTFQIHNQYTFQQFLYNNNSIMLEHGLSHMVKSFQSKQIAGRV

***** : : * * * : : * : : * : : * : : * : : * : : * : : *

COX2_MOUSE	AGGRNVPIAVQAVAKASIDQSRQMKYQSLNEYRKRFRSLKPYTSFEELTGEKEMAAELKAL
COX2_RAT	AGGRNVPIAVQAVAKASIDQSRQMKYQSLNEYRKRFRSLKPYTSFEELTGEKEMAAELKAL
COX2_GUINEAPIG	AGGRNVPLAVQAVAKASIEHSRQMKYQSLNEYRKRFRFLMKPYTSFEELTGEKEMAAELKAL
COX2_RABBIT	AGGRNVPPAVQAVAKASIDQSRQMKYQSLNEYRKRFRLLKPYTSFEELTGEKEMAAELKAL
COX2_HORSE	AGGRNVPAQAQKIAKASIDQSRQMKYQSLNEYRKRFRLLTPYKSFEEELTGEKEMAAELKAL
COX2_CANINE	AGGRNVPAVQAVAKASIDQSRQMKYQSLNEYRKRFRLLKPYTSFEELTGEKEMAAELKAL
COX2_SHEEP	AGGRNLPAAVEKVSQASIDQSRQMKYQSFNEYRKRFRLLKPYTSFEELTGEKEMAAELKAL
COX2_HUMAN	AGGRNVPPAVQAVQKVSQASIDQSRQMKYQSFNEYRKRFRMLKPYTSFEELTGEKEMAAELKAL
COX2_CHICKEN	AGGKNVPWAVQAVAKASIDQSRQMKYQSLNEYRKRFRMLKPFKSFEEELTGEKEMAAELKAL

** : : * * : : * : : * : : * : : * : : * : : * : : * : : * : : *

fig. 3D

9/10

COX2_MOUSE	YSDIDVMELYPALLVEKPRPDAIFGETMVELGAPFSLKGLMGNPICS	SPQYWKPSTFFGGEV
COX2_RAT	YHDIDAMELYPALLVEKPRPDAIFGETMVELGAPFSLKGLMGNPICS	SPQYWKPSTFFGGEV
COX2_GUINEAPIG	YGDIDAMELYPALLVEKPRPDAIFGETMVEMGAPFSLKGLMGNPICS	PHYWKPSTFFGGEV
COX2_RABBIT	YGDIDAVELYPALLVERPRPDAIFGESMVEMGAPFSLKGLMGNPICS	PNYWKPSTFFGGEV
COX2_HORSE	YGDIDAMELYPALLVEKPRPDAIFGETMVELGAPFSLKGLLGNPICS	SPDYWKPSTFFGGEV
COX2_CANINE	YGDIDAMELYPALLVEKPRPDAIFGETMVEMGAPFSLKGLMGNPICS	PDYWKPSTFFGGEV
COX2_SHEEP	YGDIDAMELYPALLVEKPPAPDAIFGETMVEAGAPFSLKGLMGNPICS	PEYWKPSTFFGGEV
COX2_HUMAN	YGDIDAMELYPGLLVEKPRPGAIFGETMVEIGAPFSLKGLMGNPICS	PEYWKPSTFFGKVK
COX2_CHICKEN	YGDIDAMELYPGLLVEKPRPGAIFGETMVEIGAPFSLKGLMGNPICS	PEYWKPSTFFGKVK

COX2_MOUSE	GFKIINTASIQSLICNNVKGCPFTSFENVQDPQTKTATINASASHSRLDDINPTVLIKRR
COX2_RAT	GFR IINTASIQSLICNNVKGCPFTSFENVQDPQTKTATINASASHSRLDDINPTVLIKRR
COX2_GUINEAPIG	GFQIVNTASIQSLICNNVKGCPVTA FNLPDQLAKTVTINASASHSRLEDLSPTVLLKGR
COX2_RABBIT	GFKIVNTASIQSLICNNVKGCPFTSFENVDPQLTKTVTINASASHSRLEDINPTVLLKGR
COX2_HORSE	GFKIINTASIQSLICNNVKGCPFTA FSVQDPQLSKAVTINASASHSGLDDVNPTVLLKER
COX2_CANINE	GFKIINTASIQSLICNNVKGCPFTA FSVQDGQLTKTVTINASSSHSGLDDINPTVLLKER
COX2_SHEEP	GFKIINTASIQSLICSNVKGCPETSFSVQDAHLTKTVTINASSSHSGLDDINPTVLLKER
COX2_HUMAN	GFQIINTASIQSLICNNVKGCPFTSFSPDPPELIKTVTINASSSRSGLDDINPTVLLKEQ
COX2_CHICKEN	GFEIINTASLQKLICNNVKGCPFTA FHLNPEPT-EATINVSTSENTAMEDINPTLLLKEQ ** * . ***** : : : : . *** * . : : : : . *** * . : : : : *

fig. 3E

10/10

COX2_MOUSE	STEL
COX2_RAT	STEL
COX2_GUINEAPIG	STEL
COX2_RABBIT	STEL
COX2_HORSE	STEL
COX2_CANINE	STEL
COX2_SHEEP	STEL
COX2_HUMAN	STEL
COX2_CHICKEN	STEL

fig. 3F

<110> Gierse, James K.
<120> Canine Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2)
<150> US 60/147,601
<160> 15
<210> 1
<211> 2587
<212> DNA
<213> Canis familiaris
<400> 1

gccgccaga gctatgagcc gggggagtcg cctgcaccgg tggccgctgc tctgtgtgt
60

gctgtgtgtg ctcccgcgc ccccggtcct gcccgcgga gcccgaccc oggcgctgt
120

gaacccgtgt tgttactacc catgtcagca ccaagggatc tgtgtccgct tggccttga
180

ccgtaccag tgtgactgca cccgcacggg ctattctggc cccaactgca ccattccga
240

gctgtggacc tggctccga attcactgcg cccagtcct tcttctctc acttctgt
300

gacgcatggg cgctggtttt ggggaattcat caatgccacc ttcattccgtg acatgtcat
360

gcgtctggta ctacagcgc gttccaacct tatcccagc cctccacct acaacatagc
420

gcatgactac atcagctggg agtccttctc caatgtgagc tattacactc gtgttctgcc
480

ctctgtgcc caagattgcc ccacgccat ggggaccaa gggaagaagc agttgccaga
540

cgcccaactc ctgggcgctc gcttctgtgt caggaggaag ttcatacctg accccaagg
600

caccaacctc atgttcgct tctttgcaca acattcacc catcagttct tcaaaacttc
660

tggcaagatg ggtctggct tcaccaaggc cttgggccat ggggtagatc ttggccacat
720

ttatggggac aatctggacc gtcagtatca gctgcggctc ttaaggatg ggaaactcaa
780

gtatcaggtt ctggatggag agatgtaccc gccatctgtg gaggaggcgc ctgtgttgat
840

gcactacca cggggcattc tgccccagag tcagatggcc gtgggccaag aagtgtttgg
900

gctgcttctt gggctcatgc tctatgccac gctctggctg cgtgagcaca atcgtgtgtg
960

tgacctgctg aaggctgagc accccacttg gggatgatgag caactcttcc agacggcccg
1020

actcatcctc attggggaga ccatcaagat tgtgattgag gagtatgtgc agcagctgag
1080

tggtacttcc ttgcagctga agttcgaccc ggagctgctg tttagcgccc agttccagta
1140

ccgcaaccgc atcgccatgg agttcaacca gctgtaccac tggcaccgc tcatgccaga
1200

ctccttcttg gtgggttccc aggagtacag ctatgagcag ttcctgttca acacctccat
1260

gctgacgcac tacgggatcg aggccctggg ggatgccttc tctcgccaga gcgcggcccg
1320

gattggtgga ggtagaaaca tagaccacca tgtcctgcac gtggctgtgg aaaccatcaa
1380

ggaatccgcg gagttgcggc tgcagccctt caatgagtac cgcaagaggt ttggcatgag
1440

gccctacatg tccttccagg aactcacagg ggagaaggag atggcagccg agttggagga
1500

gctgtatgga gacattgatg ccttgggaatt ctacccgggg cttcttcttg agaagtcca
1560

tccaaactcc atctttggag agagtatgat agaaattggg gctcccttct cccttaaggg
1620

cctcctaggg aatcccatct gttctccaga gtactggaag ccaagcacat tcggtggtga
1680

gatgggcttc aatatggtca agacagccac actgaagaag ctggtctgcc ttaacaccaa
1740

gacttgctcc tatgtttcct tccgtgtgcc tgaccccccac caggatggcg ggcttggtg
1800

ggagcggccg tccacagagc tctgaggggg cagagcagca gcattctgga ggggtgactt
1860

gtcatcccag aatgctgagg ctgggggttaa taatcccaaa tgttgggtct ttggtttgcc
1920

tcaagaatat caaggtcaac atttagaact ttgtgtctct caccattat ctggaatc
1980

atggtcttgt ttgttattct agaatgctga attcctggtt gaccatctag aatggatgga
2040

gtgatgcttc tttggcaagc cagaacactg gttcctggcc gacaacctag aatgtcagac
2100

ttctggttga cttaagacgt aggcattctc taatgtgaag ctctgacag aatcatctag
2160

aaagataggg gattcttatt ttgcattcta gaattctggg cagccctcca gcatgttgat
2220

ttttttcact ggcagttcag aatggtgtgc tcttgattgc tgatccaaaa tagtggctgg
2280

tatgccagat cagtcttgct ctgaatgcct agaatggtaa tttgattcat tttcctgttc
2340

agtgaatac ccccaaagca ggagaatcta cagcctaacc agagtgcatt gcctgcctct
2400

gtgcctgccc caaggactta gggggcagag tgttcttcct gggatgctga ctgagaccct
2460

ggtccaagga gatagaacag gtgggctttt tccaggatcat tggttggagg ccaccagagc
2520

tctgttgcca tctttgtctt gactcatgac agctgtttct catgaaacta ataaaatttt
2580

ttttccc
2587

<210> 2
<211> 608
<212> PRT
<213> Canis familiaris

<223> unsure at all Xaa locations
<400> 2

Met Ser Arg Gly Ser Arg Leu His Arg Trp Pro Leu Leu Leu Xaa Xaa
1 5 10 15

Leu Leu Xaa Leu Leu Leu Leu Pro Pro Pro Val Leu Pro Ala Glu
20 25 30

Xaa Xaa Ala Arg Thr Pro Ala Pro Val Asn Pro Cys Cys Tyr Tyr Pro
35 40 45

Cys Gln His Gln Gly Ile Cys Val Arg Phe Gly Leu Asp Arg Tyr Gln

4

50		55		60
Cys Asp Cys Thr Arg Thr Gly Tyr Ser Gly Pro Asn Cys Thr Ile Pro				
65		70		75
80				
Glu Leu Trp Thr Trp Leu Arg Asn Ser Leu Arg Pro Ser Pro Ser Phe				
	85		90	95
Leu His Phe Leu Leu Thr His Gly Arg Trp Phe Trp Glu Phe Ile Asn				
100		105		110
Ala Thr Phe Ile Arg Asp Met Leu Met Arg Leu Val Leu Thr Ala Arg				
115		120		125
Ser Asn Leu Ile Pro Ser Pro Pro Thr Tyr Asn Ile Ala His Asp Tyr				
130		135		140
Ile Ser Trp Glu Ser Phe Ser Asn Val Ser Tyr Tyr Thr Arg Val Leu				
145		150		155
160				
Pro Ser Val Pro Gln Asp Cys Pro Thr Pro Met Gly Thr Lys Gly Lys				
	165		170	175
Lys Gln Leu Pro Asp Ala Gln Leu Leu Gly Arg Arg Phe Leu Leu Arg				
180		185		190
Arg Lys Phe Ile Pro Asp Pro Gln Gly Thr Asn Leu Met Phe Ala Phe				
195		200		205
Phe Ala Gln His Phe Thr His Gln Phe Phe Lys Thr Ser Gly Lys Met				
210		215		220
Gly Pro Gly Phe Thr Lys Ala Leu Gly His Gly Val Asp Leu Gly His				
225		230		235
240				
Ile Tyr Gly Asp Asn Leu Asp Arg Gln Tyr Gln Leu Arg Leu Phe Lys				
	245		250	255
Asp Gly Lys Leu Lys Tyr Gln Val Leu Asp Gly Glu Met Tyr Pro Pro				
260		265		270
Ser Val Glu Glu Ala Pro Val Leu Met His Tyr Pro Arg Gly Ile Leu				
275		280		285
Pro Gln Ser Gln Met Ala Val Gly Gln Glu Val Phe Gly Leu Leu Pro				
290		295		300
Gly Leu Met Leu Tyr Ala Thr Leu Trp Leu Arg Glu His Asn Arg Val				
305		310		315
320				
Cys Asp Leu Leu Lys Ala Glu His Pro Thr Trp Gly Asp Glu Gln Leu				
	325		330	335
Phe Gln Thr Ala Arg Leu Ile Leu Ile Gly Glu Thr Ile Lys Ile Val				
340		345		350

Ile Glu Glu Tyr Val Gln Gln Leu Ser Gly Tyr Phe Leu Gln Leu Lys
 355 360 365
 Phe Asp Pro Glu Leu Leu Phe Ser Ala Gln Phe Gln Tyr Arg Asn Arg
 370 375 380
 Ile Ala Met Glu Phe Asn Gln Leu Tyr His Trp His Pro Leu Met Pro
 385 390 395 400
 Asp Ser Phe Trp Val Gly Ser Gln Glu Tyr Ser Tyr Glu Gln Phe Leu
 405 410 415
 Phe Asn Thr Ser Met Leu Thr His Tyr Gly Ile Glu Ala Leu Val Asp
 420 425 430
 Ala Phe Ser Arg Gln Ser Ala Gly Arg Ile Gly Gly Gly Arg Asn Ile
 435 440 445
 Asp His His Val Leu His Val Ala Val Glu Thr Ile Lys Glu Ser Arg
 450 455 460
 Glu Leu Arg Leu Gln Pro Phe Asn Glu Tyr Arg Lys Arg Phe Gly Met
 465 470 475 480
 Arg Pro Tyr Met Ser Phe Gln Glu Leu Thr Gly Glu Lys Glu Met Ala
 485 490 495
 Ala Glu Leu Glu Glu Leu Tyr Gly Asp Ile Asp Ala Leu Glu Phe Tyr
 500 505 510
 Pro Gly Leu Leu Leu Glu Lys Cys His Pro Asn Ser Ile Phe Gly Glu
 515 520 525
 Ser Met Ile Glu Ile Gly Ala Pro Phe Ser Leu Lys Gly Leu Leu Gly
 530 535 540
 Asn Pro Ile Cys Ser Pro Glu Tyr Trp Lys Pro Ser Thr Phe Gly Gly
 545 550 555 560
 Glu Met Gly Phe Asn Met Val Lys Thr Ala Thr Leu Lys Lys Leu Val
 565 570 575
 Cys Leu Asn Thr Lys Thr Cys Pro Tyr Val Ser Phe Arg Val Pro Asp
 580 585 590
 Pro His Gln Asp Gly Gly Pro Gly Val Glu Arg Pro Ser Thr Glu Leu
 595 600 605

<210> 3
 <211> 3632
 <212> DNA
 <213> Canis familiaris

<400> 3

gagcacgctc gggaactccg cacagcgctt gcctcctcca gcgccgcagc cccgagccca
60

ggacgggaac gcctccgctt cgcctccgc cgccgctcc gcctccgccc cgcctccgc
120

ctccgcccgc gcctctgcca cgcgccgcgc tccgccgcgc ccccgcccgc cgccgcgatg
180

ctggcccgcg ccctggtgct ctgcgccgc ctggcggtcg tccgcgcagc aaatccttgc
240

tgttcccacc catgtcaaaa ccaaggtatt tgtatgagca caggatttga ccagtataag
300

tgtgactgta cccgaacagg attctacggc gaaaactgct caacaccgga atttctgaca
360

agaataaaat tatacctgaa acccactcca aatacagtac actacatact taccacttc
420

aagggcgctt ggaacattgt caataacatc cccttctgc gaaatacaat tatgaaatat
480

gtgttgacat cccggtcaca ttgtattgag agtccaccaa cttataatgt gaactacggc
540

tataaaagct gggaagcctt ttctaacctc tcctattata ccagagctct tccccctgta
600

cctgatgact gtccaacacc catgggtgtg aaaggcaaga aagagcttcc tgattcaaaa
660

gagattgtgg aaaagtttct tctgcgaaga aagttcattc ctgatcccca aggcaccaat
720

atgatgtttg cattctttgc ccagcacttt acccatcaat ttttcaagac agatcataag
780

cgaggaccag ctttcaccaa aggattgggc catgggggtg acttaaatca tgtttatggg
840

gaaactttgg atagacaaca taaactgcgc cttttcaagg atggaaaaat gaaatatcag
900

gtaattgatg gagagggtga tcctctacc gtcaaagata ctcagggtcg gatgatctac
960

ccacctcatg ttctgaaca cctgcagttt gctgtgggcc aggaggtctt tgggtctggtg
1020

cctggtctga tgatgtatgc caccatttgg ctgcgggagc ataacagagt gtgtgatgtg
1080

cttaaacagg agcaccaga atgggatgat gagcggttat tccagacgag caggctaata
1140

cttataggag aaaccattaa gattgtgatt gaagactatg tacaacactt gattggctat
1200

cacttcaagc tgaagtttga cccagagctg cttttcaacc aacaattcca gtacaaaaac
1260

cgcattgctg ctgagtttaa cacactctac cactggcacc ccttcctgcc tgacaccttg
1320

caaatagatg accaggagta caatttccaa cagtttatct acaacaactc tatattattg
1380

gaacatggcc ttaccagtt tgtggaatca ttcagcaggc aaattgctgg cagggttgcc
1440

ggtggcagga atgttccagc tgcagtacaa caagtagcaa aagcttcgat tgaccagagc
1500

agacagatga aataccagtc tcttaatgag tatcgcaaac gcttaggct gaagccctat
1560

acatcattcg aagaacttac aggagagaag gaaatggctg cgggggttga ggccctttat
1620

ggtgatattg atgccatgga gctgtatcct gccctcttgg tagaaaagcc tcgtccagat
1680

gccatctttg gtgagaccat ggtagaaatg ggagcaccat tctccttgaa aggacttatg
1740

ggtaatccca tctgttcacc tgactactgg aagcctagca cctttggtgg agaagtaggc
1800

tttaaaatca tcaacactgc ctcaatccag tctctcatct gcaataacgt gaagggctgt
1860

ccattcactg cattctctgt tcaagacgga caactcacca aaacagtcac cattaatgca
1920

agctcttcgc actccggtct agatgacatc aatcccacag tctactgaa agaacggta
1980

actgaactat agaagcctgt taatcctatt tatttattta tatggacgat tcttttaac
2040

ttaattattt aatatttatg ttaaactcct tatgttactt aacatctgtt aaggagaaag
2100

gggtcatact tgtgaagatt ttcatgtcac tattttaaag atgtcttcgg gttaaagagg
2160

aaaacagttt tctgttttat aaatgagttt gacatccttt tacttgaatt tcagtttata
2220

ttattattat gaacaaaagc gaagatgttg gatatttaaa tgctgttcca ggatgacaaa
2280

atgctgcaag tttttttcga cactatcggg atttctagtg tatcttcctt ggtgcattag
2340

aagcaactac ctgcacactt tcttttcttc tgtagccac tgtgctggta gaaactctct
2400

tctgatcagt ttactttctt gtttccttga tttttaagat ctgagtatac ctttctttgg
2460

actctgtcta tactttctta cctgaacttg tgtaagtttt caggaaaacc tcaactcagg
2520

actactagta atttagctcc tcttaagagg aatgaaagga aaaaaaaaaac agcccttaaa
2580

ggactctata cacaacagta tacacttatt ttaagtgaag ggcagcgatc tttttgtaaa
2640

ttaattttta attgtaacag aaggcttcaa tgaggctaag gactgacaaa gaactgtagg
2700

gggttcttga tggaaggaag tcgtatttct attaagaata tcctttctca tttaaaagca
2760

aagtcaaatt ctgaatagtt cccgggaaga taatgtttct tttccacatc tcattgtcag
2820

ctgacatttg ctggtactgt atacttaatt tattgaggat tattatttgt tttgttagga
2880

tgttattata aactaggttt aagctgcaat cttttttttt tttttttgca ttatgtgaga
2940

atcagtatat cttctttgag attaactctg aattattatg taaacactaa gagaaattat
3000

ttgagatctg tgagtaaatt ttcaggaacc caactctggt atattgaaat atggcaagtt
3060

ggaattgaaa tatagatttc cctatatacc agccacaga gaacactgtg tctcattaac
3120

ctgcatgtac cataagatct ttacagtttt gagggaccta tggatccttc attaagtcac
3180

tcagctataa cttattgagg acacaggtgc aaatatcact gtgggtttta atattttttg
3240

tggaattctaa tttttttaaa tcatgcactg attacagata acatcagtat ttgtaaatga
3300

gaaagcatgt ctttaggtag agaaagaaaa tgaaatttta ttaaaggaaa taactcaggg
3360

gaatttttaa gattttatgt ttaaaaagtt aagaaatagt caatattaga agggcttatg
3420

taaaaacctt ttttaacttca ctgaaagaaa ttttggttatt aatatcaatg ccgactgaat
3480

tcggagtata atatatgaat gttttggtgc ctcagacaaa tgtgtattta aattaactta
3540

tgtaagatgt aagtgttacc aaatgcctgt ttatttttat actatctaag aatgaaaaat
3600

ctcttctaaa ataaattttt gcctgtttcc at
3632

<210> 4
<211> 604
<212> PRT
<213> Canis familiaris

<400> 4

Met Leu Ala Arg Ala Leu Val Leu Cys Ala Ala Leu Ala Val Val Arg
1 5 10 15

Ala Ala Asn Pro Cys Cys Ser His Pro Cys Gln Asn Gln Gly Ile Cys
20 25 30

Met Ser Thr Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
35 40 45

Phe Tyr Gly Glu Asn Cys Ser Thr Pro Glu Phe Leu Thr Arg Ile Lys
50 55 60

Leu Tyr Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
65 70 75 80

Phe Lys Gly Val Trp Asn Ile Val Asn Asn Ile Pro Phe Leu Arg Asn
85 90 95

Thr Ile Met Lys Tyr Val Leu Thr Ser Arg Ser His Leu Ile Glu Ser
100 105 110

Pro Pro Thr Tyr Asn Val Asn Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Pro Asp Asp
130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
145 150 155 160

Lys Glu Ile Val Glu Lys Phe Leu Leu Arg Arg Lys Phe Ile Pro Asp
165 170 175

Pro Gln Gly Thr Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
 180 185 190
 His Gln Phe Phe Lys Thr Asp His Lys Arg Gly Pro Ala Phe Thr Lys
 195 200 205
 Gly Leu Gly His Gly Val Asp Leu Asn His Val Tyr Gly Glu Thr Leu
 210 215 220
 Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
 225 230 235 240
 Gln Val Ile Asp Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln
 245 250 255
 Val Glu Met Ile Tyr Pro Pro His Val Pro Glu His Leu Gln Phe Ala
 260 265 270
 Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
 275 280 285
 Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
 290 295 300
 Glu His Pro Glu Trp Asp Asp Glu Arg Leu Phe Gln Thr Ser Arg Leu
 305 310 315 320
 Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
 325 330 335
 His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
 340 345 350
 Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
 355 360 365
 Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Leu Gln Ile Asp
 370 375 380
 Asp Gln Glu Tyr Asn Phe Gln Gln Phe Ile Tyr Asn Asn Ser Ile Leu
 385 390 395 400
 Leu Glu His Gly Leu Thr Gln Phe Val Glu Ser Phe Ser Arg Gln Ile
 405 410 415
 Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Ala Ala Val Gln Gln
 420 425 430
 Val Ala Lys Ala Ser Ile Asp Gln Ser Arg Gln Met Lys Tyr Gln Ser
 435 440 445
 Leu Asn Glu Tyr Arg Lys Arg Phe Arg Leu Lys Pro Tyr Thr Ser Phe
 450 455 460
 Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Gly Leu Glu Ala Leu

11

465 470 475 480
 Tyr Gly Asp Ile Asp Ala Met Glu Leu Tyr Pro Ala Leu Leu Val Glu
 485 490 495
 Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Met Gly
 500 505 510
 Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
 515 520 525
 Asp Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Lys Ile
 530 535 540
 Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
 545 550 555 560
 Cys Pro Phe Thr Ala Phe Ser Val Gln Asp Gly Gln Leu Thr Lys Thr
 565 570 575
 Val Thr Ile Asn Ala Ser Ser Ser His Ser Gly Leu Asp Asp Ile Asn
 580 585 590
 Pro Thr Val Leu Leu Lys Glu Arg Ser Thr Glu Leu
 595 600

<210> 5
 <211> 2554
 <212> DNA
 <213> Homo sapiens

<400> 5

gcgccatgag ccggagtctc ttgctccggt tcttgctggt cctgctcctg ctcccgcgc
60

tccccgtcct gctcgcggac ccaggggcgc ccacgccagt gaatccctgt tgttactatc
120

catgccagca ccagggcatc tgtgtccgct tcggccttga ccgctaccag tgtgactgca
180

cccgacggg ctattccggc cccaactgca ccacccctgg cctgtggacc tggtccgga
240

attcactgcg gccagcccc tctttcaccc atttctgct cactcacggg cgctgggttct
300

gggagtttgt caatgccacc ttcacccgag agatgctcat gcgcctggta ctacagtg
360

gtccaacct tatccccagt cccccacct acaactcagc acatgactac atcagctggg
420

12

agtctttctc caacgtgagc tattacactc gtattctgcc ctctgtgect aaagattgcc
480

ccacacccat gggaaccaa gggaagaagc agttgccaga tgcccagctc ctggcccgcc
540

gcttcctgct caggaggaag ttcatacctg accccaagg caccaacctc atgtttgect
600

tctttgcaca acattcacc caccagttct tcaaaacttc tggcaagatg ggtcctggct
660

tcaccaaggc cttgggcat ggggtagacc tcggccacat ttatggagac aatctggagc
720

gtcagtatca actgcggtc tttaaggatg ggaaactcaa gtaccagggtg ctggatggag
780

aaatgtaccc gccctcggtg gaagaggcgc ctgtgttgat gcactacccc cgaggcatcc
840

cgccccagag ccagatggct gtgggccagg aggtgtttgg gctgcttcct gggctcatgc
900

tgtatgccac gctctggcta cgtgagcaca accgtgtgtg tgacctgctg aaggctgagc
960

acccacctg gggcgatgag cagcttttcc agacgacccg cctcatcctc ataggggaga
1020

ccatcaagat tgtcatcgag gagtacgtgc agcagctgag tggctatttc ctgcagctga
1080

aatttgaccc agagctgctg ttcggtgtcc agttccaata ccgcaaccgc attgccatgg
1140

agttcaacca tctctaccac tggcaccccc tcatgcctga ctccttcaag gtgggctccc
1200

aggagtacag ctacgagcag ttctgtttca acacctccat gttggtggac tatggggttg
1260

aggccctggt ggatgccttc tctcgccaga ttgctggccg gatcggtggg ggcaggaaca
1320

tggaccacca catcctgcat gtggctgtgg atgtcatcag ggagtctcgg gagatgcggc
1380

tcgagccctt caatgagtac cgcaagaggt ttggcatgaa accctacacc tccttcagg
1440

agctcgtagg agagaaggag atggcagcag agttggagga attgtatgga gacattgatg
1500

cgttggagtt ctaccctgga ctgcttcttg aaaagtgcc tccaaactct atctttgggg
1560

agagtatgat agagattggg gctccctttt cctcaaggg tctcctaggg aatccccatct
1620

gttctccgga gtactggaag ccgagcacat ttggcggcga ggtgggcttt aacattgtca
1680

agacggccac actgaagaag ctggtctgcc tcaacaccaa gacctgtccc tacgtttcct
1740

tccgtgtgcc ggatgccagt caggatgatg ggcctgctgt ggagcgacca tccacagagc
1800

tctgaggggc aggaaagcag cattctggag gggagagctt tgtgcttgc attccagagt
1860

gctgaggcca gggctgatgg tcttaaatgc tcattttctg gtttggcatg gtgagtgttg
1920

gggttgacat ttagaacttt aagtctcacc cattatctgg aatattgtga ttctgtttat
1980

tcttcagaa tgctgaactc cttgttagcc cttcagattg ttaggagtgg ttctcatttg
2040

gtctgccaga atactgggtt cttagttagc aacctagaat gtcagatttc tggttgattt
2100

gtaacacagt cattctagga tgtggagcta ctgatgaaat ctgctagaaa gttaggggggt
2160

tcttattttg cattccagaa tcttgacttt ctgattgggtg attcaaagtg ttgtgttccc
2220

tggctgatga tccagaacag tggctcgtat cccaaatctg tcagcatctg gctgtctaga
2280

atgtggattt gattcatttt cctgttcagt gagatatcat agagacggag atcctaagggt
2340

ccaacaagaa tgcattccct gaatctgtgc ctgcactgag agggcaagga agtgggggtg
2400

tcttcttggg acccccacta agaccctgggt ctgaggatgt agagagaaca ggtgggctgt
2460

attcacgcca ttggttgga gctaccagag ctctatcccc atccagggtct tgactcatgg
2520

cagctgtttc tcatgaagct aataaaattc gccc
2554

<210> 6
<211> 608
<212> PRT

14

<213> Homo sapiens

<223> unsure at all Xaa locations

<400> 6

Met Ser Arg Xaa Ser Xaa Xaa Xaa Xaa Xaa Xaa Leu Leu Leu Arg Phe
 1 5 10 15
 Leu Leu Phe Leu Leu Leu Leu Pro Pro Leu Pro Val Leu Leu Ala Asp
 20 25 30
 Pro Gly Ala Pro Thr Xaa Xaa Pro Val Asn Pro Cys Cys Tyr Tyr Pro
 35 40 45
 Cys Gln His Gln Gly Ile Cys Val Arg Phe Gly Leu Asp Arg Tyr Gln
 50 55 60
 Cys Asp Cys Thr Arg Thr Gly Tyr Ser Gly Pro Asn Cys Thr Ile Pro
 65 70 75 80
 Gly Leu Trp Thr Trp Leu Arg Asn Ser Leu Arg Pro Ser Pro Ser Phe
 85 90 95
 Thr His Phe Leu Leu Thr His Gly Arg Trp Phe Trp Glu Phe Val Asn
 100 105 110
 Ala Thr Phe Ile Arg Glu Met Arg Met Arg Leu Val Leu Thr Val Arg
 115 120 125
 Ser Asn Leu Ile Pro Ser Pro Pro Thr Tyr Asn Ser Ala His Asp Tyr
 130 135 140
 Ile Ser Trp Glu Ser Phe Ser Asn Val Ser Tyr Tyr Thr Arg Ile Leu
 145 150 155 160
 Pro Ser Val Pro Lys Asp Cys Pro Thr Pro Met Gly Thr Lys Gly Lys
 165 170 175
 Lys Gln Leu Pro Asp Ala Gln Leu Leu Ala Arg Arg Phe Leu Leu Arg
 180 185 190
 Arg Lys Phe Ile Pro Asp Pro Gln Gly Thr Asn Leu Met Phe Ala Phe
 195 200 205
 Phe Ala Gln His Phe Thr His Gln Phe Phe Lys Thr Ser Gly Lys Met
 210 215 220
 Gly Pro Gly Phe Thr Lys Ala Leu Gly His Gly Val Asp Leu Gly His
 225 230 235 240
 Ile Tyr Gly Asp Asn Leu Glu Arg Gln Tyr Gln Leu Arg Leu Phe Lys
 245 250 255
 Asp Gly Lys Leu Lys Tyr Gln Val Leu Asp Gly Glu Met Tyr Pro Pro
 260 265 270

15

Ser Val Glu Glu Ala Pro Val Leu Met His Tyr Pro Arg Gly Ile Pro
 275 280 285

Pro Gln Ser Gln Met Ala Val Gly Gln Glu Val Phe Gly Leu Leu Pro
 290 295 300

Gly Leu Met Leu Tyr Ala Thr Leu Trp Leu Arg Glu His Asn Arg Val
 305 310 315 320

Cys Asp Leu Leu Lys Ala Glu His Pro Thr Trp Gly Asp Glu Gln Leu
 325 330 335

Phe Gln Thr Thr Arg Leu Ile Leu Ile Gly Glu Thr Ile Lys Ile Val
 340 345 350

Ile Glu Glu Tyr Val Gln Gln Leu Ser Gly Tyr Phe Leu Gln Leu Lys
 355 360 365

Phe Asp Pro Glu Leu Leu Phe Gly Val Gln Phe Gln Tyr Arg Asn Arg
 370 375 380

Ile Ala Met Glu Phe Asn His Leu Tyr His Trp His Pro Leu Met Pro
 385 390 395 400

Asp Ser Phe Lys Val Gly Ser Gln Glu Tyr Ser Tyr Glu Gln Phe Leu
 405 410 415

Phe Asn Thr Ser Met Leu Val Asp Tyr Gly Val Glu Ala Leu Val Asp
 420 425 430

Ala Phe Ser Arg Gln Ile Ala Gly Arg Ile Gly Gly Gly Arg Asn Met
 435 440 445

Asp His His Ile Leu His Val Ala Val Asp Val Ile Arg Glu Ser Arg
 450 455 460

Glu Met Arg Leu Gln Pro Phe Asn Glu Tyr Arg Lys Arg Phe Gly Met
 465 470 475 480

Lys Pro Tyr Thr Ser Phe Gln Glu Leu Val Gly Glu Lys Glu Met Ala
 485 490 495

Ala Glu Leu Glu Glu Leu Tyr Gly Asp Ile Asp Ala Leu Glu Phe Tyr
 500 505 510

Pro Gly Leu Leu Leu Glu Lys Cys His Pro Asn Ser Ile Phe Gly Glu
 515 520 525

Ser Met Ile Glu Ile Gly Ala Pro Phe Ser Leu Lys Gly Leu Leu Gly
 530 535 540

Asn Pro Ile Cys Ser Pro Glu Tyr Trp Lys Pro Ser Thr Phe Gly Gly
 545 550 555 560

Glu Val Gly Ph Asn Ile Val Lys Thr Ala Thr Leu Lys Lys Leu Val
 565 570 575

Cys Leu Asn Thr Lys Thr Cys Pro Tyr Val Ser Phe Arg Val Pro Asp
 580 585 590

Ala Ser Gln Asp Asp Gly Pro Ala Val Glu Arg Pro Ser Thr Glu Leu
 595 600 605

<210> 7
 <211> 3387
 <212> DNA
 <213> Homo sapiens

<400> 7

gtccaggaac tctcagcag cgctccttc agtccacag ccagacgcc tcagacagca
 60

aagcctaccc ccgcgcgcg cctgcccgc cgctgcgatg ctgcccgcg cctgctgct
 120

gtgcgcggtc ctggcgctca gccatacagc aaatccttgc tgttcccacc catgtcaaaa
 180

ccgaggtgta tgtatgagtg tgggatttga ccagtataag tgcgattgta ccgggacagg
 240

attctatgga gaaaactgct caacaccgga atttttgaca agaataaaat tatttctgaa
 300

accactcca aacacagtgc actacatact taccacttc aagggtattt ggaacgttgt
 360

gaataacatt cccttccttc gaaatgcaat tatgagttat gtgttgacat ccagatcaca
 420

tttgattgac agtcaccaa cttacaatgc tgactatggc tacaaaagct gggaagcctt
 480

ctctaacctc tcctattata ctagagccct tcctcctgtg cctgatgatt gcccgactcc
 540

cttgggtgtc aaaggtaaaa agcagcttcc tgattcaaat gagattgtgg gaaaattgct
 600

tctaagaaga aagttcatcc ctgatcccca gggtcaaac atgatgtttg cattctttgc
 660

ccagcacttc acgcatcagt ttttcaagac agatcataag cgagggccag ctttcaccaa
 720

cgggctgggc catggggtgg acttaaatca tatttacggt gaaactctgg ctagacagcg
 780

taaactgcgc cttttcaagg atggaaaaat gaaatatcag ataattgatg gagagatgta
 840

tcctcccaca gtcaaagata ctcaggcaga gatgatctac cctcctcaag tccctgagca
900

tctacggttt gctgtggggc aggaggtctt tggctcgggtg cctgggtctga tgatgtatgc
960

cacaatctgg ctgagggaac acaacagagt atgcgatgtg cttaaacagg agcatcctga
1020

atgggggtgat gagcagttgt tccagacaag caggctaata ctgataggag agactattaa
1080

gattgtgatt gaagattatg tgcaacactt gagtggctat cacttcaaac tgaaatttga
1140

cccagaacta cttttcaaca aacaattcca gtaccaaagt cgtattgctg ctgaatttaa
1200

cacctctat cactggcatc cccttctgcc tgacacctt caaattcatg accagaaata
1260

caactatcaa cagtttatct acaacaactc tatattgctg gaacatggaa ttaccagtt
1320

tgttgaatca ttcaccaggc aaattgctgg cagggttgct ggtggtagga atgttcacc
1380

cgcagtacag aaagtatcac aggcttccat tgaccagagc aggagatga aataccagtc
1440

ttttaatgag taccgcaaac gctttatgct gaagccctat gaatcatttg aagaacttac
1500

aggagaaaag gaaatgtctg cagagttgga agcactctat ggtgacatcg atgctgtgga
1560

gctgtatcct gcccttctgg tagaaaagcc tcggccagat gccatctttg gtgaaacct
1620

ggtagaagtt ggagcaccat tctccttgaa aggacttatg ggtaatgtta tatgttctcc
1680

tgctactgg aagccaagca cttttggtgg agaagtgggt tttcaaatca tcaacactgc
1740

ctcaattcag tctctcatct gcaataacgt gaagggtgt ccctttactt cattcagtgt
1800

tccagatcca gagctcatta aaacagtcac catcaatgca agttcttccc gctccggact
1860

agatgatatc aatcccacag tactactaaa agaacgttcg actgaactgt agaagtctaa
1920

tgatcatatt tatttattta tatgaacat gtctattaat ttaattattt aataatattt
1980

atattaaact ccttatgtta cttaacatct tctgtaacag aagtcagtac tcctgttgcg
2040

gagaaaggag tcatacttgt gaagactttt atgtcactac tctaaagatt ttgctgttgc
2100

tgtaaagttt ggaaaacagt ttttattctg ttttataaac cagagagaaa tgagttttga
2160

cgtcttttta cttgaatttc aacttatatt ataaggacga aagtaaagat gtttgaatac
2220

ttaaacacta tcacaagatg ccaaaatgct gaaagttttt acactgtcga tgtttccaat
2280

gcatcttcca tgatgcatta gaagtaacta atgtttgaaa ttttaaagta cttttgggta
2340

tttttctgtc atcaaacaaa acaggtatca gtgcattatt aaatgaatat ttaaattaga
2400

cattaccagt aatttcatgt ctacttttta aaatcagcaa tgaaacaata atttgaaatt
2460

tctaaattca tagggtagaa tcacctgtaa aagcttgttt gatttcttaa agttattaaa
2520

cttgtaacata taccaaaaag aagctgtctt ggatttaaat ctgtaaaatc agatgaaatt
2580

ttactacaat tgcttgtaa aatattttat aagtgatgtt cctttttcac caagagtata
2640

aaccttttta gtgtgactgt taaaacttcc ttttaaatca aaatgccaaa tttattaagg
2700

tggtggagcc actgcagtgt tatctcaaaa taagaatata ctgttgagat attccagaat
2760

ctgtttatat ggctggtaac atgtaaaaac ccataaccc cgccaaaagg ggtcctaccc
2820

ttgaacataa agcaataacc aaaggagaaa agcccaaatt attggttcca aatttaggg
2880

ttaaactttt tgaagcaaac ttttttttag ccttgtgcac tgcagacctg gtactcagat
2940

tttgetatga ggttaatgaa gtaccaagct gtgcttgaat aacgatatgt tttctcagat
3000

tttctgttgt acagttaaatt ttagcagtc atatacatt gcaaaagtag caatgacctc
3060

ataaaatacc tcttcaaaat gcttaaattc atttcacaca ttaattttat ctcaagtcttg
3120

aagccaattc agtaggtgca ttggaatcaa gcctggctac ctgcatgctg ttcctttttct
3180

tttctttctt tagccatttt gctaagagac acagtcttct caaacacttc gtttctccta
3240

ttttgtttta ctagttttta gatcagagtt cactttcttt ggactctgcc tatattttct
3300

tacctgaact ttgcaagtt ttcaggtaaa cctcagctca ggactgctat ttagctcctc
3360

ttaagaagat taaaaaaaaa aaaaaag
3387

<210> 8
<211> 604
<212> PRT
<213> Homo sapiens

<400> 8

Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Val Leu Ala Leu Ser His
1 5 10 15

Thr Ala Asn Pro Cys Cys Ser His Pro Cys Gln Asn Arg Gly Val Cys
20 25 30

Met Ser Val Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
35 40 45

Phe Tyr Gly Glu Asn Cys Ser Thr Pro Glu Phe Leu Thr Arg Ile Lys
50 55 60

Leu Phe Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
65 70 75 80

Phe Lys Gly Phe Trp Asn Val Val Asn Asn Ile Pro Phe Leu Arg Asn
85 90 95

Ala Ile Met Ser Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser
100 105 110

Pro Pro Thr Tyr Asn Ala Asp Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Pro Asp Asp
130 135 140

Cys Pro Thr Pro Leu Gly Val Lys Gly Lys Lys Gln Leu Pro Asp Ser
145 150 155 160

20

Asn Glu Ile Val Glu Lys Leu Leu Leu Arg Arg Lys Phe Ile Pro Asp
 165 170 175
 Pro Gln Gly Ser Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
 180 185 190
 His Gln Phe Phe Lys Thr Asp His Lys Arg Gly Pro Ala Phe Thr Asn
 195 200 205
 Gly Leu Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu
 210 215 220
 Ala Arg Gln Arg Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
 225 230 235 240
 Gln Ile Ile Asp Gly Glu Met Tyr Pro Pro Thr Val Lys Asp Thr Gln
 245 250 255
 Ala Glu Met Ile Tyr Pro Pro Gln Val Pro Glu His Leu Arg Phe Ala
 260 265 270
 Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
 275 280 285
 Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
 290 295 300
 Glu His Pro Glu Trp Gly Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu
 305 310 315 320
 Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
 325 330 335
 His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
 340 345 350
 Phe Asn Lys Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
 355 360 365
 Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile His
 370 375 380
 Asp Gln Lys Tyr Asn Tyr Gln Gln Phe Ile Tyr Asn Asn Ser Ile Leu
 385 390 395 400
 Leu Glu His Gly Ile Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile
 405 410 415
 Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Pro Ala Val Gln Lys
 420 425 430
 Val Ser Gln Ala Ser Ile Asp Gln Ser Arg Gln Met Lys Tyr Gln Ser
 435 440 445
 Phe Asn Glu Tyr Arg Lys Arg Phe Met Leu Lys Pro Tyr Glu Ser Phe

21

450 455 460
 Glu Glu Leu Thr Gly Glu Lys Glu Met Ser Ala Glu Leu Glu Ala Leu
 465 470 475 480
 Tyr Gly Asp Ile Asp Ala Val Glu Leu Tyr Pro Ala Leu Leu Val Glu
 485 490 495
 Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Val Gly
 500 505 510
 Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Val Ile Cys Ser Pro
 515 520 525
 Ala Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Gln Ile
 530 535 540
 Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
 545 550 555 560
 Cys Pro Phe Thr Ser Phe Ser Val Pro Asp Pro Glu Leu Ile Lys Thr
 565 570 575
 Val Thr Ile Asn Ala Ser Ser Ser Arg Ser Gly Leu Asp Asp Ile Asn
 580 585 590
 Pro Thr Val Leu Leu Lys Glu Arg Ser Thr Glu Leu
 595 600

<210> 9
 <211> 604
 <212> PRT
 <213> Mus musculus
 <400> 9

Met Leu Phe Arg Ala Val Leu Leu Cys Ala Ala Leu Gly Leu Ser Gln
 1 5 10 15
 Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Glu Cys
 20 25 30
 Met Ser Thr Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
 35 40 45
 Phe Tyr Gly Glu Asn Cys Thr Thr Pro Glu Phe Leu Thr Arg Ile Lys
 50 55 60
 Leu Leu Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
 65 70 75 80
 Phe Lys Gly Val Trp Asn Ile Val Asn Asn Ile Pro Phe Leu Arg Ser
 85 90 95
 Leu Thr Met Lys Tyr Val Leu Thr Ser Arg Ser Tyr Leu Ile Asp Ser

22

100	105	110
Pro Pro Thr Tyr Asn Val His Tyr Gly Tyr Lys Ser Trp Glu Ala Phe		
115	120	125
Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Asp		
130	135	140
Cys Pro Thr Pro Met Gly Val Lys Gly Asn Lys Glu Leu Pro Asp Ser		
145	150	155
Lys Glu Val Leu Glu Lys Val Leu Leu Arg Arg Glu Phe Ile Pro Asp		
165	170	175
Pro Gln Gly Ser Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr		
180	185	190
His Gln Phe Phe Lys Thr Asp His Lys Arg Gly Pro Gly Phe Thr Arg		
195	200	205
Gly Leu Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu		
210	215	220
Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Leu Lys Tyr		
225	230	235
Gln Val Ile Gly Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln		
245	250	255
Val Glu Met Ile Tyr Pro Pro His Ile Pro Glu Asn Leu Gln Phe Ala		
260	265	270
Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala		
275	280	285
Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Ile Leu Lys Gln		
290	295	300
Glu His Pro Glu Trp Gly Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu		
305	310	315
Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln		
325	330	335
His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu		
340	345	350
Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ser Glu Phe Asn		
355	360	365
Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Asn Ile Glu		
370	375	380
Asp Gln Glu Tyr Ser Phe Lys Gln Phe Leu Tyr Asn Asn Ser Ile Leu		
385	390	395
		400

23

Leu	Glu	His	Gly	Leu	Thr	Gln	Phe	Val	Glu	Ser	Phe	Thr	Arg	Gln	Ile	
405								410							415	
Ala	Gly	Arg	Val	Ala	Gly	Gly	Arg	Asn	Val	Pro	Ile	Ala	Val	Gln	Ala	
420						425					430					
Val	Ala	Lys	Ala	Ser	Ile	Asp	Gln	Ser	Arg	Glu	Met	Lys	Tyr	Gln	Ser	
435				440				445								
Leu	Asn	Glu	Tyr	Arg	Lys	Arg	Phe	Ser	Leu	Lys	Pro	Tyr	Thr	Ser	Phe	
450				455				460								
Glu	Glu	Leu	Thr	Gly	Glu	Lys	Glu	Met	Ala	Ala	Glu	Leu	Lys	Ala	Leu	
465				470				475				480				
Tyr	Ser	Asp	Ile	Asp	Val	Met	Glu	Leu	Tyr	Pro	Ala	Leu	Leu	Val	Glu	
485					490					495						
Lys	Pro	Arg	Pro	Asp	Ala	Ile	Phe	Gly	Glu	Thr	Met	Val	Glu	Leu	Gly	
500				505				510								
Ala	Pro	Phe	Ser	Leu	Lys	Gly	Leu	Met	Gly	Asn	Pro	Ile	Cys	Ser	Pro	
515				520				525								
Gln	Tyr	Trp	Lys	Pro	Ser	Thr	Phe	Gly	Gly	Glu	Val	Gly	Phe	Lys	Ile	
530				535				540								
Ile	Asn	Thr	Ala	Ser	Ile	Gln	Ser	Leu	Ile	Cys	Asn	Asn	Val	Lys	Gly	
545				550				555				560				
Cys	Pro	Phe	Thr	Ser	Phe	Asn	Val	Gln	Asp	Pro	Gln	Pro	Thr	Lys	Thr	
565					570					575						
Ala	Thr	Ile	Asn	Ala	Ser	Ala	Ser	His	Ser	Arg	Leu	Asp	Asp	Ile	Asn	
580				585				590								
Pro	Thr	Val	Leu	Ile	Lys	Arg	Arg	Ser	Thr	Glu	Leu					
595				600												

<210>	10
<211>	604
<212>	PRT
<213>	Rattus norvegicus
<400>	10

Met Leu Phe Arg Ala Val Leu Leu Cys Ala Ala Leu Ala Leu Ser His
1 5 10 15

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Glu Cys
20 25 30

Met Ser Ile Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
35 40 45

24

Phe Tyr Gly Glu Asn Cys Thr Thr Pro Glu Ile Leu Thr Arg Ile Lys
 50 55 60

Leu Leu Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
 65 70 75 80

Phe Lys Gly Val Trp Asn Ile Val Asn Asn Ile Pro Phe Leu Arg Asn
 85 90 95

Ser Ile Met Arg Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser
 100 105 110

Pro Pro Thr Tyr Asn Val His Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
 115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Asp
 130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Asn Lys Glu Leu Pro Asp Ser
 145 150 155 160

Lys Glu Val Leu Glu Lys Val Leu Leu Arg Arg Glu Phe Ile Pro Asp
 165 170 175

Pro Gln Gly Thr Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
 180 185 190

His Gln Phe Phe Lys Thr Asp Gln Lys Arg Gly Pro Gly Phe Thr Arg
 195 200 205

Gly Leu Gly His Gly Val Asp Leu Asn His Val Tyr Gly Glu Thr Leu
 210 215 220

Asp Arg Gln His Lys Leu Arg Leu Phe Gln Asp Gly Lys Leu Lys Tyr
 225 230 235 240

Gln Val Ile Gly Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln
 245 250 255

Val Asp Met Ile Tyr Pro Pro His Val Pro Glu His Leu Arg Phe Ala
 260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
 275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Ile Leu Lys Gln
 290 295 300

Glu His Pro Glu Trp Asp Asp Glu Arg Leu Phe Gln Thr Ser Arg Leu
 305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
 325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
 340 345 350

25

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ser Glu Phe Asn
 355 360 365
 Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Asn Ile Glu
 370 375 380
 Asp Gln Glu Tyr Thr Phe Lys Gln Phe Leu Tyr Asn Asn Ser Ile Leu
 385 390 395 400
 Leu Glu His Gly Leu Ala His Phe Val Glu Ser Phe Thr Arg Gln Ile
 405 410 415
 Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Ile Ala Val Gln Ala
 420 425 430
 Val Ala Lys Ala Ser Ile Asp Gln Ser Arg Glu Met Lys Tyr Gln Ser
 435 440 445
 Leu Asn Glu Tyr Arg Lys Arg Phe Ser Leu Lys Pro Tyr Thr Ser Phe
 450 455 460
 Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Lys Ala Leu
 465 470 475 480
 Tyr His Asp Ile Asp Ala Met Glu Leu Tyr Pro Ala Leu Leu Val Glu
 485 490 495
 Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Leu Gly
 500 505 510
 Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
 515 520 525
 Gln Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Arg Ile
 530 535 540
 Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
 545 550 555 560
 Cys Pro Phe Ala Ser Phe Asn Val Gln Asp Pro Gln Pro Thr Lys Thr
 565 570 575
 Ala Thr Ile Asn Ala Ser Ala Ser His Ser Arg Leu Asp Asp Ile Asn
 580 585 590
 Pro Thr Val Leu Ile Lys Arg Arg Ser Thr Glu Leu
 595 600

<210> 11
 <211> 604
 <212> PRT
 <213> Cavia porcellus
 <400> 11

Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Ala Leu Ala Leu Gly Gln
 1 5 10 15
 Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Glu Cys
 20 25 30
 Leu Ser Val Gly Phe Asp Arg Tyr Lys Cys Asp Cys Thr Arg Thr Gly
 35 40 45
 Tyr Tyr Gly Glu Asn Cys Thr Thr Pro Glu Phe Leu Thr Arg Ile Lys
 50 55 60
 Leu Leu Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
 65 70 75 80
 Phe Lys Gly Val Trp Asn Ile Val Asn Asn Ile Pro Phe Leu Arg Asn
 85 90 95
 Ala Ile Met Ile Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser
 100 105 110
 Pro Pro Thr Tyr Asn Ala His Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
 115 120 125
 Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Asp
 130 135 140
 Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
 145 150 155 160
 Asn Glu Val Leu Glu Lys Val Leu Leu Arg Arg Lys Phe Ile Pro Asp
 165 170 175
 Pro Gln Gly Thr Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
 180 185 190
 His Gln Phe Phe Lys Ser Asp Gln Lys Arg Gly Pro Ala Phe Thr Thr
 195 200 205
 Gly Leu Ala His Gly Val Asp Leu Ser His Ile Tyr Gly Glu Thr Leu
 210 215 220
 Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
 225 230 235 240
 Gln Ile Ile Asp Gly Glu Met Tyr Pro Pro Thr Val Lys Glu Thr Gln
 245 250 255
 Val Glu Met Met Tyr Pro Pro Tyr Ile Pro Glu His Ala Arg Phe Ala
 260 265 270
 Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
 275 280 285
 Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln

27

290	295	300
Glu His Pro Glu Trp Asp 305	Asp Glu Arg Leu Phe 310	Gln Thr Ser Arg Leu 315 320
Ile Leu Ile Gly Glu Thr 325	Ile Lys Ile Val Ile 330	Glu Asp Tyr Val Gln 335
His Leu Ser Gly Tyr His 340	Phe Lys Leu Lys Phe 345	Asp Pro Glu Leu Leu 350
Phe Asn Gln Gln Phe Gln 355	Tyr Gln Asn Arg Ile Ala 360	Ser Glu Phe Asn 365
Thr Leu Tyr His Trp His 370	Pro Leu Leu Pro Asp 375	Thr Phe Gln Ile Asp 380
Asp Gln Val Tyr Asn Phe 385	Gln Gln Phe Leu Tyr 390	Asn Asn Ser Ile Leu 395 400
Val Glu His Gly Leu Thr 405	Gln Phe Val Glu Ser 410	Phe Thr Lys Gln Ile 415
Ala Gly Arg Val Ala Gly 420	Gly Arg Asn Val Pro 425	Leu Ala Val Gln Arg 430
Val Ala Lys Ala Ser Ile 435	Glu His Ser Arg Lys 440	Met Lys Tyr Gln Ser 445
Leu Asn Glu Tyr Arg Lys 450	Arg Phe Leu Met Lys 455	Pro Tyr Thr Ser Phe 460
Glu Glu Leu Thr Gly Glu 465	Lys Glu Met Ala Ala 470	Gly Leu Glu Ala Leu 475 480
Tyr Gly Asp Ile Asp Ala 485	Met Glu Leu Tyr Pro 490	Ala Leu Leu Val Glu 495
Lys Pro Arg Pro Asp Ala 500	Ile Phe Gly Glu Thr 505	Met Val Glu Met Gly 510
Ala Pro Phe Ser Leu Lys 515	Gly Leu Met Gly Asn 520	Pro Ile Cys Ser Pro 525
His Tyr Trp Lys Pro Ser 530	Thr Phe Gly Gly Glu 535	Val Gly Phe Gln Ile 540
Val Asn Thr Ala Ser Ile 545	Gln Ser Leu Ile Cys 550	Asn Asn Val Lys Gly 555 560
Cys Pro Val Thr Ala Phe 565	Asn Leu Pro Asp Pro 570	Gln Leu Ala Lys Thr 575
Val Thr Ile Asn Ala Ser 580	Ala Ser His Ser Arg 585	Leu Glu Asp Leu Ser 590

28

Pro Thr Val Leu Leu Lys Gly Arg Ser Thr Glu Leu
 595 600

<210> 12
 <211> 604
 <212> PRT
 <213> Oryctolagus cuniculus

<400> 12

Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Ala Val Ala Leu Ser His
 1 5 10 15

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Val Cys
 20 25 30

Met Thr Met Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
 35 40 45

Phe Tyr Gly Glu Asn Cys Ser Thr Pro Glu Phe Leu Thr Arg Ile Lys
 50 55 60

Leu Leu Leu Lys Pro Thr Pro Asp Thr Val His Tyr Ile Leu Thr His
 65 70 75 80

Phe Lys Gly Val Trp Asn Ile Val Asn Ser Ile Pro Phe Leu Arg Asn
 85 90 95

Ser Ile Met Lys Tyr Val Leu Thr Ser Arg Ser His Met Ile Asp Ser
 100 105 110

Pro Pro Thr Tyr Asn Val His Tyr Asn Tyr Lys Ser Trp Glu Ala Phe
 115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Asp
 130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
 145 150 155 160

Lys Asp Val Val Glu Lys Leu Leu Leu Arg Arg Lys Phe Ile Pro Asp
 165 170 175

Pro Gln Gly Thr Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
 180 185 190

His Gln Phe Phe Lys Thr Asp Leu Lys Arg Gly Pro Ala Phe Thr Lys
 195 200 205

Gly Leu Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu
 210 215 220

Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
 225 230 235 240

29

Gln Val Ile Asp Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln
 245 250 255
 Val Glu Met Ile Tyr Pro Pro His Ile Pro Ala His Leu Gln Phe Ala
 260 265 270
 Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
 275 280 285
 Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
 290 295 300
 Glu His Pro Glu Trp Asp Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu
 305 310 315 320
 Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
 325 330 335
 His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
 340 345 350
 Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
 355 360 365
 Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile Asp
 370 375 380
 Asp Gln Gln Tyr Asn Tyr Gln Gln Phe Leu Tyr Asn Asn Ser Ile Leu
 385 390 395 400
 Leu Glu His Gly Leu Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile
 405 410 415
 Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Pro Ala Val Gln Lys
 420 425 430
 Val Ala Lys Ala Ser Ile Asp Gln Ser Arg Gln Met Lys Tyr Gln Ser
 435 440 445
 Leu Asn Glu Tyr Arg Lys Arg Phe Leu Leu Lys Pro Tyr Glu Ser Phe
 450 455 460
 Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Ala Leu
 465 470 475 480
 Tyr Gly Asp Ile Asp Ala Val Glu Leu Tyr Pro Ala Leu Leu Val Glu
 485 490 495
 Arg Pro Arg Pro Asp Ala Ile Phe Gly Glu Ser Met Val Glu Met Gly
 500 505 510
 Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
 515 520 525
 Asn Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Lys Ile
 530 535 540

Val Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
545 550 555 560

Cys Pro Phe Thr Ser Phe Asn Val Pro Asp Pro Gln Leu Thr Lys Thr
565 570 575

Val Thr Ile Asn Ala Ser Ala Ser His Ser Arg Leu Glu Asp Ile Asn
580 585 590

Pro Thr Val Leu Leu Lys Gly Arg Ser Thr Glu Leu
595 600

<210> 13
<211> 604
<212> PRT
<213> Equus caballus

<400> 13

Met Leu Ala Arg Ala Leu Leu Leu Cys Val Ala Leu Ala Leu Gly His
1 5 10 15

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Val Cys
20 25 30

Met Ser Val Gly Phe Asp Gln Tyr Gln Cys Asp Cys Thr Arg Thr Gly
35 40 45

Phe Tyr Gly Glu Asn Cys Ser Thr Pro Glu Phe Leu Thr Arg Ile Lys
50 55 60

Leu Phe Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
65 70 75 80

Phe Lys Gly Val Trp Asn Ile Val Asn Ser Phe Pro Phe Leu Arg Asn
85 90 95

Ala Val Met Lys Tyr Val Leu Val Ser Arg Ser His Leu Ile Glu Ser
100 105 110

Pro Pro Thr Tyr Asn Ala Gln Tyr Gly Tyr Lys Ser Trp Glu Ser Phe
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Gly
130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
145 150 155 160

Lys Glu Ile Val Glu Lys Phe Leu Leu Arg Arg Lys Phe Ile Pro Asp
165 170 175

Pro Gln Gly Thr Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
180 185 190

His Gln Phe Phe Lys Thr Asp Pro Lys Arg Gly Pro Ala Phe Thr Lys
 195 200 205
 Gly Leu Gly His Gly Val Asp Leu Ser His Ile Tyr Gly Glu Thr Leu
 210 215 220
 Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
 225 230 235 240
 Gln Ile Ile Asn Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln
 245 250 255
 Val Glu Met Ile Tyr Pro Pro His Ile Pro Glu His Leu Arg Phe Ala
 260 265 270
 Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
 275 280 285
 Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
 290 295 300
 Glu His Pro Glu Trp Asp Asp Glu Arg Leu Phe Gln Thr Ser Arg Leu
 305 310 315 320
 Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
 325 330 335
 His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
 340 345 350
 Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
 355 360 365
 Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile Asp
 370 375 380
 Asp Gln Glu Tyr Asn Phe Gln Gln Phe Leu Tyr Asn Asn Ser Ile Leu
 385 390 395 400
 Leu Glu His Gly Leu Thr Gln Phe Val Glu Ser Phe Ser Arg Gln Ile
 405 410 415
 Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Ala Ala Ala Gln Lys
 420 425 430
 Ile Ala Lys Ala Ser Ile Asp Gln Ser Arg Glu Met Lys Tyr Gln Ser
 435 440 445
 Leu Asn Glu Tyr Arg Lys Arg Phe Arg Leu Thr Pro Tyr Lys Ser Phe
 450 455 460
 Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Ala Leu
 465 470 475 480
 Tyr Gly Asp Ile Asp Ala Met Glu Leu Tyr Pro Ala Leu Leu Val Glu

32

	485		490		495
Lys Pro Arg	Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Leu Gly				
	500		505		510
Ala Pro Phe Ser Leu Lys Gly Leu Leu Gly Asn Pro Ile Cys Ser Pro					
	515		520		525
Asp Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Lys Ile					
	530		535		540
Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly					
	545		550		555
Cys Pro Phe Thr Ala Phe Ser Val Gln Asp Pro Gln Leu Ser Lys Ala					
		565	570		575
Val Thr Ile Asn Ala Ser Ala Ser His Ser Gly Leu Asp Asp Val Asn					
	580		585		590
Pro Thr Val Leu Leu Lys Glu Arg Ser Thr Glu Leu					
	595		600		

<210> 14
 <211> 604
 <212> PRT
 <213> Ovis aries

<223> unsure at all Xaa locations
 <400> 14

Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Ala Val Val Cys Gly Xaa					
1	5		10		15
Ala Ala Asn Pro Cys Cys Ser His Pro Cys Gln Asn Arg Gly Val Cys					
	20		25		30
Met Ser Val Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly					
	35		40		45
Phe Tyr Gly Glu Asn Cys Thr Thr Pro Glu Phe Leu Thr Arg Ile Lys					
	50		55		60
Leu Leu Leu Lys Pro Thr Pro Asp Thr Val His Tyr Ile Leu Thr His					
	65		70		75
Phe Lys Gly Val Trp Asn Ile Val Asn Lys Ile Ser Phe Leu Arg Asn					
		85	90		95
Met Ile Met Arg Tyr Val Leu Thr Ser Arg Ser His Leu Ile Glu Ser					
	100		105		110
Pro Pro Thr Tyr Asn Val His Tyr Ser Tyr Lys Ser Trp Glu Ala Phe					
	115		120		125

33

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Pro Asp Asp
 130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Arg Lys Glu Leu Pro Asp Ser
 145 150 155 160

Lys Glu Val Val Lys Lys Val Leu Leu Arg Arg Lys Phe Ile Pro Asp
 165 170 175

Pro Gln Gly Thr Asn Leu Met Phe Ala Phe Phe Ala Gln His Phe Thr
 180 185 190

His Gln Phe Phe Lys Thr Asp Ile Glu Arg Gly Pro Ala Phe Thr Lys
 195 200 205

Gly Lys Asn His Gly Val Asp Leu Ser His Val Tyr Gly Glu Ser Leu
 210 215 220

Glu Arg Gln His Asn Arg Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
 225 230 235 240

Gln Met Ile Asn Gly Glu Met Tyr Pro Pro Thr Val Lys Asp Thr Gln
 245 250 255

Val Glu Met Ile Tyr Pro Pro His Ile Pro Glu His Leu Lys Phe Ala
 260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
 275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
 290 295 300

Glu His Pro Glu Trp Gly Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu
 305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
 325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
 340 345 350

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
 355 360 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Val Phe Gln Ile Asp
 370 375 380

Gly Gln Glu Tyr Asn Tyr Gln Gln Phe Ile Tyr Asn Asn Ser Val Leu
 385 390 395 400

Leu Glu His Gly Val Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile
 405 410 415

Ala Gly Arg Val Ala Gly Arg Arg Asn Leu Pro Ala Ala Val Glu Lys
 420 425 430

Val Ser Lys Ala Ser Leu Asp Gln Ser Arg Glu Met Lys Tyr Gln Ser
435 440 445

Phe Asn Glu Tyr Arg Lys Arg Phe Leu Leu Lys Pro Tyr Glu Ser Phe
450 455 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Ala Leu
465 470 475 480

Tyr Gly Asp Ile Asp Ala Met Glu Leu Tyr Pro Ala Leu Leu Val Glu
485 490 495

Lys Pro Ala Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Ala Gly
500 505 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
515 520 525

Glu Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Lys Ile
530 535 540

Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Ser Asn Val Lys Gly
545 550 555 560

Cys Pro Phe Thr Ser Phe Ser Val Gln Asp Ala His Leu Thr Lys Thr
565 570 575

Val Thr Ile Asn Ala Ser Ser Ser His Ser Gly Leu Asp Asp Ile Asn
580 585 590

Pro Thr Val Leu Leu Lys Glu Arg Ser Thr Glu Leu
595 600

<210> 15
<211> 604
<212> PRT
<213> Gallus gallus

<223> unsure at all Xaa locations
<400> 15

Met Leu Leu Pro Cys Ala Leu Leu Ala Ala Leu Leu Ala Ala Gly His
1 5 10 15

Ala Ala Asn Pro Cys Cys Ser Leu Pro Cys Gln Asn Arg Gly Val Cys
20 25 30

Met Thr Thr Gly Phe Asp Arg Tyr Glu Cys Asp Cys Thr Arg Thr Gly
35 40 45

Tyr Tyr Gly Glu Asn Cys Thr Thr Pro Glu Phe Phe Thr Trp Leu Lys
50 55 60

Leu Ile Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His

35

65		70		75		80
Phe Lys Gly Val Trp Asn Ile Ile Asn Asn Ile Ser Phe Leu Arg Asp						
	85			90		95
Thr Ile Met Arg Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser						
	100			105		110
Pro Pro Thr Tyr Asn Ser Asp Tyr Ser Tyr Lys Ser Trp Glu Ala Tyr						
	115			120		125
Ser Asn Leu Ser Tyr Tyr Thr Arg Ser Leu Pro Pro Val Gly His Asp						
	130			135		140
Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser						
	145			150		155
Lys Leu Ile Val Glu Lys Phe Leu Leu Arg Arg Lys Phe Ile Pro Asp						
	165			170		175
Pro Gln Gly Thr Asn Val Met Phe Thr Phe Phe Ala Gln His Phe Thr						
	180			185		190
His Gln Phe Phe Lys Thr Asp His Lys Lys Gly Pro Gly Phe Thr Lys						
	195			200		205
Ala Tyr Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu						
	210			215		220
Glu Arg Gln Leu Lys Leu Arg Leu Arg Lys Asp Gly Lys Leu Lys Tyr						
	225			230		235
Gln Met Ile Asp Gly Glu Met Tyr Pro Pro Thr Val Lys Asp Thr Gln						
	245			250		255
Ala Glu Met Ile Tyr Pro Pro His Val Pro Glu His Leu Gln Phe Ser						
	260			265		270
Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala						
	275			280		285
Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln						
	290			295		300
Glu His Pro Glu Trp Asp Asp Glu Gln Leu Phe Gln Thr Thr Arg Leu						
	305			310		315
Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln						
	325			330		335
His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu						
	340			345		350
Phe Asn Gln Arg Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn						
	355			360		365

36

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile His
 370 375 380

Asn Gln Glu Tyr Thr Phe Gln Gln Phe Leu Tyr Asn Asn Ser Ile Met
 385 390 395 400

Leu Glu His Gly Leu Ser His Met Val Lys Ser Phe Ser Lys Gln Ser
 405 410 415

Ala Gly Arg Val Ala Gly Gly Lys Asn Val Pro Ala Ala Val Gln Lys
 420 425 430

Val Ala Lys Ala Ser Ile Asp Gln Ser Arg Gln Met Arg Tyr Gln Ser
 435 440 445

Leu Asn Glu Tyr Arg Lys Arg Phe Met Leu Lys Pro Phe Lys Ser Phe
 450 455 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Glu Leu
 465 470 475 480

Tyr Gly Asp Ile Asp Ala Met Glu Leu Tyr Pro Gly Leu Leu Val Glu
 485 490 495

Lys Pro Arg Pro Gly Ala Ile Phe Gly Glu Thr Met Val Glu Ile Gly
 500 505 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Thr Ile Cys Ser Pro
 515 520 525

Glu Tyr Trp Lys Pro Ser Thr Phe Gly Gly Lys Val Gly Phe Glu Ile
 530 535 540

Ile Asn Thr Ala Ser Leu Gln Lys Leu Ile Cys Asn Asn Val Lys Gly
 545 550 555 560

Cys Pro Phe Thr Ala Phe His Val Leu Asn Pro Glu Pro Thr Xaa Glu
 565 570 575

Ala Thr Ile Asn Val Ser Thr Ser Asn Thr Ala Met Glu Asp Ile Asn
 580 585 590

Pro Thr Leu Leu Leu Lys Glu Gln Ser Ala Glu Leu
 595 600

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/19565

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N9/02 C12Q1/26 C07K16/00 C12N5/10 C12Q1/68
G01N33/68 C12N15/62 A61K48/00 A61K38/43

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EMBL, BIOSIS, MEDLINE, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>COYNE D W ET AL: "REGULATION OF EICOSANOID BIOSYNTHESIS BY PHORBOL ESTER IN MADIN DARBY CANINE KIDNEY CELLS" AMERICAN JOURNAL OF PHYSIOLOGY, vol. 259, no. 4 PART 2, 1990, pages F698-F703, XP000942451 ISSN: 0002-9513 Abstract page F699, right-hand column, line 37 —page F699, right-hand column, line 56; figure 5</p> <p style="text-align: center;">— -/-</p>	1-15,31, 32



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

A document member of the same patent family

Date of the actual completion of the international search

15 Dec mb r 2000

Date of mailing of the international search report

12.01.01

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tr. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Meyer, W

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/19565

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>BEVERELLI F ET AL: "Chronic inhibition of NO synthase enhances the production of prostacyclin in coronary arteries through upregulation of the cyclooxygenase type 1 isoform."</p> <p>FUNDAMENTAL & CLINICAL PHARMACOLOGY, vol. 11, no. 3, 1997, pages 252-259, XP000942441</p> <p>ISSN: 0767-3981</p> <p>abstract</p> <p>page 256, left-hand column, line 1 -page 256, left-hand column, line 13</p>	1-15,31, 32
X	<p>SMITH CAROLYN J ET AL: "Reduced gene expression of endothelial nitric oxide synthase and cyclooxygenase-1 in canine heart failure."</p> <p>CIRCULATION, vol. 90, no. 4 PART 2, 1994, page I299</p> <p>XP000942524</p> <p>67th Scientific Sessions of the American Heart Association; Dallas, Texas, USA; November 14-17, 1994</p> <p>ISSN: 0009-7322</p> <p>abstract</p>	1-15,31, 32
A	<p>THOMAS P S ET AL: "PROSTAGLANDIN D-2 PRODUCTION AND IDENTIFICATION OF PROSTAGLANDIN H SYNTHASE WITHIN CANINE MAST CELL GRANULE"</p> <p>AMERICAN JOURNAL OF PHYSIOLOGY, vol. 263, no. 2 PART 1, 1992, pages L168-L176, XP000942450</p> <p>ISSN: 0002-9513</p> <p>Abstract</p> <p>page L173, right-hand column, line 4 -page L173, right-hand column, line 14</p>	1-15,31, 32
X	<p>RICKETTS AP. ET AL.: "Evaluation of selective inhibition of canine cyclooxygenase 1 and 2 by carprofen and other nonsteroidal anti-inflammatory drugs 'see comments!'"</p> <p>AM J VET RES 1998 NOV;59(11):1441-6, XP000942455</p> <p>abstract</p> <p>page 1441, right-hand column, paragraph 2</p>	1-15,31, 32
A	<p>EP 0 736 769 A (MERCK FROSST CANADA INC) 9 October 1996 (1996-10-09)</p> <p>the whole document</p>	1-15,31, 32
A	<p>WO 94 06919 A (UNIV ROCHESTER) 31 March 1994 (1994-03-31)</p> <p>the whole document</p>	1-15,31, 32
	-/-	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/19565

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TAKAHASHI Y. ET AL.: "Immunoaffinity purification and cDNA cloning of human platelet prostaglandin endoperoxide synthase (cyclooxygenase)." BIOCHEM BIOPHYS RES COMMUN 1992 JAN 31;182(2):433-8, XP000941369 abstract; figure 1	1-15,31, 32
X	SCHIAFFINO M VITTORIA ET AL: "Cloning of a human homologue of the Xenopus laevis APX gene from the ocular albinism type 1 critical region." HUMAN MOLECULAR GENETICS, vol. 4, no. 3, 1995, pages 373-382, XP002155571 ISSN: 0964-6906	24,25, 27-30
Y	abstract	31-67
X	OSUKA KOJI ET AL: "Inducible cyclooxygenase expression in canine basilar artery after experimental subarachnoid hemorrhage." STROKE, vol. 29, no. 6, June 1998 (1998-06), pages 1219-1222, XP000942445 ISSN: 0039-2499	16-30
Y	discussion abstract; figure 2	31-67
X	OSUKA KOJI ET AL: "Vasodilator effects on canine basilar artery induced by intracisternal interleukin-1beta." JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM, vol. 17, no. 12, December 1997 (1997-12), pages 1337-1345, XP000942453 ISSN: 0271-678X	16-30
Y	abstract; figure 8	31-67
Y	WO 96 40720 A (UNIV ROCHESTER) 19 December 1996 (1996-12-19) claims 1-57	31-67
P,X	DATABASE EMBL 'Online! Accession No. AC046189, 14 April 2000 (2000-04-14) BIRREN B ET AL.: "Homo sapiens chromosome 4, clone RP11-356M17" XP002155572 abstract	16-23

-/-

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/19565

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>HILDEBRAND J D ET AL: "SHROOM, A PDZ DOMAIN-CONTAINING ACTIN-BINDING PROTEIN, IS REQUIRED FOR NEURAL TUBE MORPHOGENESIS IN MICE" CELL, CELL PRESS, CAMBRIDGE, NA, US, vol. 99, no. 5, 24 November 1999 (1999-11-24), pages 485-497, XP000941350 ISSN: 0092-8674 abstract; figure 4</p>	16-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/19565

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-15, 31 and 32

Claims relating to Seq. Id. No. 1, 2, functional variants thereof and the use thereof

2. Claims: 16-30 and 33-67

Claims relating to Seq. Id. No. 3, 4, functional variants thereof and the use thereof

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 24 and 25 relate to a protein defined by its corresponding DNA sequence. The corresponding sequence (SEQ ID: 4) is not a DNA sequence and thus the subject-matter of present claim is not clear within the meaning of Article 6 PCT.

However, the subject-matter of said claims has been interpreted as relating to a protein encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 3 or its complement.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/19565

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0736769 A	09-10-1996	US 5543297 A	06-08-1996
		AT 155811 T	15-08-1997
		AU 5690694 A	19-07-1994
		CA 2151231 A	07-07-1994
		WO 9414977 A	07-07-1994
		DE 69312513 D	28-08-1997
		DE 69312513 T	15-01-1998
		DK 675964 T	15-09-1997
		EP 0675964 A	11-10-1995
		ES 2105611 T	16-10-1997
		GR 3025111 T	30-01-1998
		JP 8504584 T	21-05-1996
WO 9406919 A	31-03-1994	AT 179756 T	15-05-1999
		AU 688282 B	12-03-1998
		AU 5165293 A	12-04-1994
		DE 69324820 D	10-06-1999
		DE 69324820 T	13-01-2000
		EP 0667911 A	23-08-1995
		JP 8501690 T	27-02-1996
		NZ 256776 A	24-10-1997
		US 5837479 A	17-11-1998
		US 6048850 A	11-04-2000
WO 9640720 A	19-12-1996	AU 6029396 A	30-12-1996
		US 5837479 A	17-11-1998
		US 6048850 A	11-04-2000